APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: NEMATODE PHOSPHOETHANOLAMINE N-METHYLTRANSFERASE-LIKE SEQUENCES

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Nematode Phosphoethanolamine N-Methyltransferase-like Sequences

CLAIM OF PRIORITY

This application claims priority under 35 USC §119(e) to U.S. Provisional Patent Application Serial No. 60/390,490, filed on June 21, 2002, the entire contents of which are hereby incorporated by reference.

BACKGROUND

Nematodes (derived from the Greek word for thread) are active, flexible, elongate, organisms that live on moist surfaces or in liquid environments, including films of water within soil and moist tissues within other organisms. While only 20,000 species of nematode have been identified, it is estimated that 40,000 to 10 million actually exist. Some species of nematodes have evolved to be very successful parasites of both plants and animals and are responsible for significant economic losses in agriculture and livestock and for morbidity and mortality in humans (Whitehead (1998) *Plant Nematode Control.* CAB International, New York).

Nematode parasites of plants can inhabit all parts of plants, including roots, developing flower buds, leaves, and stems. Plant parasites are classified on the basis of their feeding habits into the broad categories: migratory ectoparasites, migratory endoparasites, and sedentary endoparasites. Sedentary endoparasites, which include the root knot nematodes (*Meloidogyne*) and cyst nematodes (*Globodera* and *Heterodera*) induce feeding sites and establish long-term infections within roots that are often very damaging to crops (Whitehead, *supra*). It is estimated that parasitic nematodes cost the horticulture and agriculture industries in excess of $78 billion worldwide a year, based on an estimated average 12% annual loss spread across all major crops. For example, it is estimated that nematodes cause soybean losses of approximately $3.2 billion annually worldwide (Barker et al. (1994) *Plant and Soil Nematodes: Societal Impact and Focus for the Future.* The Committee on National Needs and Priorities in Nematology. Cooperative State Research Service, US Department of Agriculture and Society of Nematologists). Several factors make the need for safe and effective nematode controls urgent. Continuing population growth, famines, and environmental degradation have heightened concern for the sustainability of

agriculture, and new government regulations may prevent or severely restrict the use of many available agricultural anthelmintic agents.

There are a very small array of chemicals available to control nematodes (Becker (1999) *Agricultural Research Magazine* 47(3):22-24; US Pat. Nos. 6,048,714). Nevertheless, the application of chemical nematicides remains the major means of nematode control. In general, chemical nematicides are highly toxic compounds known to cause substantial environmental damage and are increasingly restricted in the amounts and locations in which then can be used. For example, the soil fumigant methyl bromide which has been used effectively to reduce nematode infestations in a variety of specialty crops, is regulated under the U.N. Montreal Protocol as an ozone-depleting substance and is scheduled for elimination in 2005 in the US (Carter (2001) *California Agriculture*, 55(3):2). It is expected that strawberry and other commodity crop industries will be significantly impacted if a suitable replacement for methyl bromide is not found. Similarly, broad-spectrum nematicides such as Telone (various formulations of 1,3-dichloropropene) have significant restrictions on their use because of toxicological concerns (Carter (2001) *California Agriculture*, Vol. 55(3):12-18).

The macrocyclic lactones (e.g., avermectins and milbemycins) and delta-toxins from *Bacillus thuringiensis* (*Bt*) are chemicals that in principle provide excellent specificity and efficacy and should allow environmentally safe control of plant parasitic nematodes. Unfortunately, in practice, these two nematicidal agents have proven less effective in agricultural applications against root pathogens. Although certain avermectins show exquisite activity against plant parasitic nematodes these chemicals are hampered by poor bioavailability due to their light sensitivity, degradation by soil microorganisms and tight binding to soil particles (Lasota & Dybas (1990) *Acta Leiden* 59(1-2):217-225; Wright & Perry (1998) Musculature and Neurobiology. In: The Physiology and Biochemistry of Free-Living and Plant-parasitic Nematodes (eds R.N. Perry & D.J. Wright), CAB International 1998). Consequently despite years of research and extensive use against animal parasitic nematodes, mites and insects (plant and animal applications), macrocyclic lactones (e.g., avermectins and milbemycins) have never been commercially developed to control plant parasitic nematodes in the soil.

Bt delta toxins must be ingested to affect their target organ, the brush border of midgut epithelial cells (Marroquin et al. (2000) *Genetics*. 155(4):1693-1699). Consequently they are not anticipated to be effective against the dispersal, non-feeding, juvenile stages of plant parasitic nematodes in the field. Because juvenile stages only commence feeding when a susceptible host has been infected, nematicides may need to penetrate the plant cuticle to be effective. Transcuticular uptake of a 65-130 kDa protein - the size of typical *Bt* delta toxins - is unlikely. Furthermore, soil mobility is expected to be relatively poor. Even transgenic approaches are hampered by the size of *Bt* delta toxins because delivery *in planta* is likely to be constrained by the exclusion of large particles by the feeding tubes of certain plant parasitic nematodes such as *Heterodera* (Atkinson et al. (1998) Engineering resistance to plant-parasitic nematodes. In: The Physiology and Biochemistry of Free-Living and Plant-parasitic Nematodes (eds R.N. Perry & D.J. Wright), CAB International 1998).

Fatty acids are a class of natural compounds that have been investigated as alternatives to the toxic, non-specific organophosphate, carbamate and fumigant pesticides (Stadler et al. (1994) *Planta Medica* 60(2):128-132; US Pat. Nos. 5,192,546; 5,346,698; 5,674,897; 5,698,592; 6,124,359). It has been suggested that fatty acids derive their pesticidal effects by adversely interfering with the nematode cuticle or hypodermis via a detergent (solubilization) effect, or through direct interaction of the fatty acids and the lipophilic regions of target plasma membranes (Davis et al. (1997) *Journal of Nematology* 29(4S):677-684). In view of this predicted mode of action it is not surprising that fatty acids are used in a variety of pesticidal applications including as herbicides (e.g., SCYTHE by Dow Agrosciences is the C9 saturated fatty acid pelargonic acid), bactericides and fungicides (US Pat. Nos. 4,771,571; 5,246,716) and insecticides (e.g., SAFER INSECTICIDAL SOAP by Safer, Inc.).

The phytotoxicity of fatty acids has been a major constraint on their general use in post-plant agricultural applications (US Pat. No. 5,093,124) and the mitigation of these undesirable effects while preserving pesticidal activity is a major area of research. Post-plant applications are desirable because of the relatively short half-life of fatty acids under field conditions.

The esterification of fatty acids can significantly decrease their phytotoxicity (US Pat. Nos. 5,674,897; 5,698,592; 6,124,359). Such modifications can however lead to loss of

nematicidal activity as is seen for linoleic, linolenic and oleic acid (Stadler et al. (1994) *Planta Medica* 60(2):128-132) and it may be impossible to completely decouple the phytotoxicity and nematicidal activity of pesticidal fatty acids because of their non-specific mode of action. Perhaps not surprisingly, the nematicidal fatty acid pelargonic acid methyl ester (US Pat. Nos. 5,674,897; 5,698,592; 6,124,359) shows a relatively small "therapeutic window" between the onset of pesticidal activity and the observation of significant phytotoxicity (Davis et al. (1997) *J Nematol* 29(4S):677-684). This is the expected result if both the phytotoxicity and the nematicidial activity derive from the non-specific disruption of plasma membrane integrity.

Ricinoleic acid, the major component of castor oil, has been shown to have an inhibitory effect on water and electrolyte absorption using everted hamster jejunal and ileal segments (Gaginella et al. (1975) *J Pharmacol Exp Ther* 195(2):355-61) and to be cytotoxic to isolated intestinal epithelial cells (Gaginella et al. (1977) *J Pharmacol Exp Ther* 201(1):259-66). These features are likely the source of the laxative properties of castor oil which is given as a purgative in humans and livestock (e.g., castor oil is a component of some de-worming protocols because of its laxative properties). In contrast, the methyl ester of ricinoleic acid is ineffective at suppressing water absorption in the hamster model (Gaginella et al. (1975) *J Pharmacol Exp Ther* 195(2):355-61).

Many plant species are known to be highly resistant to nematodes. The best documented of these include marigolds (*Tagetes* spp.), rattlebox (*Crotalaria spectabilis*), chrysanthemums (*Chrysanthemum* spp.), castor bean (*Ricinus communis*), margosa (*Azardiracta indica*), and many members of the family *Asteraceae* (family *Compositae*) (Hackney & Dickerson. (1975) *J Nematol* 7(1):84-90). In the case of the *Asteraceae*, the photodynamic compound alpha-terthienyl has been shown to account for the strong nematicidal activity of the roots. Castor beans are plowed under as a green manure before a seed crop is set. However, a significant drawback of the castor plant is that the seed contains toxic compounds (such as ricin) that can kill humans, pets, and livestock and is also highly allergenic. In many cases however, the active principle(s) for plant nematicidal activity has not been discovered and it remains difficult to derive commercially successful nematicidal products from these resistant plants or to transfer the resistance to agronomically important crops such as soybeans and cotton.

Genetic resistance to certain nematodes is available in some commercial cultivars (e.g., soybeans), but these are restricted in number and the availability of cultivars with both desirable agronomic features and resistance is limited. The production of nematode resistant commercial varieties by conventional plant breeding based on genetic recombination through sexual crosses is a slow process and is often further hampered by a lack of appropriate germplasm.

There remains an urgent need to develop environmentally safe, target-specific ways of controlling plant parasitic nematodes. In the specialty crop markets, economic hardship resulting from nematode infestation is highest in strawberries, bananas, and other high value vegetables and fruits. In the high-acreage crop markets, nematode damage is greatest in soybeans and cotton. There are however, dozens of additional crops that suffer from nematode infestation including potato, pepper, onion, citrus, coffee, sugarcane, greenhouse ornamentals and golf course turf grasses.

Nematode parasites of vertebrates (e.g., humans, livestock and companion animals) include gut roundworms, hookworms, pinworms, whipworms, and filarial worms. They can be transmitted in a variety of ways, including by water contamination, skin penetration, biting insects, or by ingestion of contaminated food.

In domesticated animals, nematode control or "de-worming" is essential to the economic viability of livestock producers and is a necessary part of veterinary care of companion animals. Parasitic nematodes cause mortality in animals (e.g., heartworm in dogs and cats) and morbidity as a result of the parasites' inhibiting the ability of the infected animal to absorb nutrients. The parasite-induced nutrient deficiency leads to disease and stunted growth in livestock and companion animals. For instance, in cattle and dairy herds, a single untreated infection with the brown stomach worm can permanently restrict an animal's ability to convert feed into muscle mass or milk.

Two factors contribute to the need for novel anthelmintics and vaccines to control animal parasitic nematodes. First, some of the more prevalent species of parasitic nematodes of livestock are building resistance to the anthelmintic drugs available currently, meaning that these products will eventually lose their efficacy. These developments are not surprising because few effective anthelmintic drugs are available and most have been used continuously. Some parasitic species have developed resistance to most of the anthelmintics

(Geents et al. (1997) *Parasitology Today* 13:149-151; Prichard (1994) *Veterinary Parasitology* 54:259-268). The fact that many of the anthelmintic drugs have similar modes of action complicates matters, as the loss of sensitivity of the parasite to one drug is often accompanied by side resistance – that is, resistance to other drugs in the same class (Sangster & Gill (1999) *Parasitology Today* 15(4):141-146). Secondly, there are some issues with toxicity for the major compounds currently available.

Infections by parasitic nematode worms result in substantial human mortality and morbidity, especially in tropical regions of Africa, Asia, and the Americas. The World Health Organization estimates 2.9 billion people are infected, and in some areas, 85% of the population carries worms. While mortality is rare in proportion to infections, morbidity is substantial and rivals diabetes and lung cancer in worldwide disability adjusted life year (DALY) measurements.

Examples of human parasitic nematodes include hookworms, filarial worms, and pinworms. Hookworms (1.3 billion infections) are the major cause of anemia in millions of children, resulting in growth retardation and impaired cognitive development. Filarial worm species invade the lymphatics, resulting in permanently swollen and deformed limbs (elephantiasis), and the eyes, causing African river blindness. The large gut roundworm *Ascaris lumbricoides* infects more than one billion people worldwide and causes malnutrition and obstructive bowel disease. In developed countries, pinworms are common and often transmitted through children in daycare.

Even in asymptomatic parasitic infections, nematodes can still deprive the host of valuable nutrients and increase the ability of other organisms to establish secondary infections. In some cases, infections can cause debilitating illnesses and can result in anemia, diarrhea, dehydration, loss of appetite, or death.

Despite some advances in drug availability and public health infrastructure and the near elimination of one tropical nematode (the water-borne Guinea worm), most nematode diseases have remained intractable problems. Treatment of hookworm diseases with anthelmintic drugs, for instance, has not provided adequate control in regions of high incidence because rapid re-infection occurs after treatment. In fact, over the last 50 years, while nematode infection rates have fallen in the United States, Europe, and Japan, the overall number of infections worldwide has kept pace with the growing world population.

Large scale initiatives by regional governments, the World Health Organization, foundations, and pharmaceutical companies are now underway attempting to control nematode infections with currently available tools, including three programs for control of Onchocerciasis (river blindness) in Africa and the Americas using ivermectin and vector control; The Global Alliance to Eliminate Lymphatic Filariasis using DEC, albendazole, and ivermectin; and the highly successful Guinea Worm Eradication Program. Until safe and effective vaccines are discovered to prevent parasitic nematode infections, anthelmintic drugs will continue to be used to control and treat nematode parasitic infections in both humans and domestic animals.

Finding effective compounds and vaccines against parasitic nematodes has been complicated by the fact that the parasites have not been amenable to culturing in the laboratory. Parasitic nematodes are often obligate parasites (i.e., they can only survive in their respective hosts, such as in plants, animals, and/or humans) with slow generation times. Thus, they are difficult to grow under artificial conditions, making genetic and molecular experimentation difficult or impossible. To circumvent these limitations, scientists have used *Caenorhabidits elegans* as a model system for parasitic nematode discovery efforts.

C. elegans is a small free-living bacteriovorous nematode that for many years has served as an important model system for multicellular animals (Burglin (1998) *Int. J. Parasitol.* 28(3):395-411). The genome of *C. elegans* has been completely sequenced and the nematode shares many general developmental and basic cellular processes with vertebrates (Ruvkin et al. (1998) *Science* 282:2033-41). This, together with its short generation time and ease of culturing, has made it a model system of choice for higher eukaryotes (Aboobaker et al. (2000) *Ann. Med.* 32:23-30).

Although *C. elegans* serves as a good model system for vertebrates, it is an even better model for study of parasitic nematodes, as *C. elegans* and other nematodes share unique biological processes not found in vertebrates. For example, unlike vertebrates, nematodes produce and use chitin, have gap junctions comprised of innexin rather than connexin and contain glutamate-gated chloride channels rather than glycine-gated chloride channels (Bargmann (1998) *Science* 282:2028-33). The latter property is of particular relevance given that the avermectin class of drugs is thought to act at glutamate-gated chloride receptors and is highly selective for invertebrates (Martin (1997) *Vet. J.* 154:11-34).

A subset of the genes involved in nematode-specific processes will be conserved in nematodes and absent or significantly diverged from homologues in other phyla. In other words, it is expected that at least some of the genes associated with functions unique to nematodes will have restricted phylogenetic distributions. The completion of the *C. elegans* genome project and the growing database of expressed sequence tags (ESTs) from numerous nematodes facilitate identification of these "nematode-specific" genes. In addition, conserved genes involved in nematode-specific processes are expected to retain the same or very similar functions in different nematodes. This functional equivalence has been demonstrated in some cases by transforming *C. elegans* with homologous genes from other nematodes (Kwa et al. (1995) *J. Mol. Biol.* 246:500-10; Redmond et al. (2001) *Mol. Biochem. Parasitol.* 112:125-131). This sort of functional conservations has also been shown in cross phyla comparisons for conserved genes and is expected to be more robust among species within a phylum. Consequently, *C. elegans* and other free-living nematode species are likely excellent surrogates for parasitic nematodes with respect to conserved nematode processes.

Many expressed genes in *C. elegans* and certain genes in other free-living nematodes can be "knocked out" genetically by a process referred to as RNA interference (RNAi), a technique that provides a powerful experimental tool for the study of gene function in nematodes (Fire et al. (1998) *Nature* 391(6669):806-811; Montgomery et al. (1998) *Proc. Natl. Acad Sci USA* 95(26):15502-15507). Treatment of a nematode with double-stranded RNA of a selected gene can destroy expressed sequences corresponding to the selected gene thus reducing expression of the corresponding protein. By preventing the translation of specific proteins, their functional significance and essentiality to the nematode can be assessed. Determination of essential genes and their corresponding proteins using *C. elegans* as a model system will assist in the rational design of anti-parasitic nematode control products.

SUMMARY

The invention features nucleic acid molecules encoding *Ascaris suum*, *Haemonchus contortus*, *Meloidogyne incognita*, *Meloidogyne javanica* and *Strongyloides stercoralis* phosphoethanolamine n-methyltransferase-like (PEAMT-like) proteins. *A. suum* is the large

roundworm of pigs and is closely related to *Ascaris lumbricoides*, a major human pathogen. *H. contortus* is a parasite of ruminants (sheep, goats, cattle and other wild ruminants) leading to emaciation, anemia and in certain cases death. As such it represents a major economic scourge. *M. javanica* and *M. incognita* are Root Knot Nematodes that cause substantial damage to several crops, including cotton, tobacco, pepper, and tomato. *S. stercoralis* is a nematode parasite that infects humans, primates, and dogs. It is one of the few nematodes that can multiply within its host and can multiply unchecked in immunosuppressed individuals.

The PEAMT-like nucleic acids and polypeptides of the invention allow for the identification of nematode species. The nucleic acids and polypeptides of the invention also allow for the identification of compounds that bind to or alter the activity of PEAMT-like polypeptides. Such compounds may provide a means for combating diseases and infestations caused by nematodes, particularly those caused by *A. suum* in pigs, *A. lumbricoides* in humans and other ascarid species in a variety of animals, *H. contortus* in ruminants, *M. javanica* and *M. incognita* (e.g., in tobacco, cotton, pepper, or tomato plants) and *S. stercoralis* (e.g., in humans, primates and dogs).

The invention is based, in part, on the identification of a cDNA encoding *A. suum* PEAMT1 (SEQ ID NO: 1). This 1786 nucleotide cDNA has a 1380 nucleotide open reading frame (SEQ ID NO: 13) encoding a 460 amino acid polypeptide (SEQ ID NO: 7).

The invention is also based, in part, on the identification of a cDNA encoding *H. contortus* PEAMT1 (SEQ ID NO: 2). This 1669 nucleotide cDNA has a 1380 nucleotide open reading frame (SEQ ID NO: 14) encoding a 460 amino acid polypeptide (SEQ ID NO: 8).

The invention is also based, in part, on the identification of a cDNA encoding *M. incognita* PEAMT1 (SEQ ID NO: 3). This 1472 nucleotide cDNA has a 1371 nucleotide open reading frame (SEQ ID NO: 15) encoding a 457 amino acid polypeptide (SEQ ID NO: 9).

The invention is based, in part, on the identification of a cDNA encoding *S. stercoralis* PEAMT1 (SEQ ID NO: 4). This 1580 nucleotide cDNA has a 1407 nucleotide open reading frame (SEQ ID NO: 16) encoding a 469 amino acid polypeptide (SEQ ID NO: 10).

The invention is also based, in part, on the identification of a cDNA encoding *A. suum* PEAMT2 (SEQ ID NO: 5). This 1533 nucleotide cDNA has a 1311 nucleotide open reading frame (SEQ ID NO: 17) encoding a 437 amino acid polypeptide (SEQ ID NO: 11).

The invention is based, in part, on the identification of a cDNA encoding *M. javanica* PEAMT2 (SEQ ID NO: 6). This 1534 nucleotide cDNA has a 1416 nucleotide open reading frame (SEQ ID NO: 18) encoding a 472 amino acid polypeptide (SEQ ID NO: 12).

In one aspect, the invention features novel nematode phosphoethanolamine n-methyltransferase-like polypeptides. Such polypeptides include purified polypeptides having the amino acid sequences set forth in SEQ ID NO: 7, 8, 9, 10, 11 or 12. Also included are polypeptides having an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 98% identical to SEQ ID NO: 7, 8, 9, 10, 11 and/or 12 as well as polypeptides having a sequence that differs from that of SEQ ID NO: 7, 8, 9, 10, 11 or 12 at 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues (amino acids). The purified polypeptides can be encoded by a nematode gene, e.g., a nematode gene other than *C. elegans*. For example, the purified polypeptide has a sequence other than SEQ ID NO: 19, 20 or 21 (*C. elegans* PEAMT1-like or PEAMT2-like proteins). The purified polypeptides can further include a heterologous amino acid sequence, e.g., an amino-terminal or carboxy-terminal sequence. Also featured are purified polypeptide fragments of the aforementioned PEAMT-like polypeptides, e.g., a fragment of at least about 20, 30, 40, 50, 75, 85, 104, 106, 113 150, 200, 250, 300, 350, 400, 450 or 470 amino acids. Non-limiting examples of such fragments include: fragments from about amino acid 1 to 50, 1 to 75, 1 to 89, 1 to 91, 1 to 99, 1 to 100, 1 to 125, 51 to 113, 93 to 104, 99 to 113, 93 to 106, 228 to 262, 250 to 280, 290 to 322, and 317 to 352 of SEQ ID NO: 7, 8, 9, 10, 11 and 12. The polypeptide or fragment thereof can be modified, e.g., processed, truncated, modified (e.g. by glycosylation, phosphorylation, acetylation, myristylation, prenylation, palmitoylation, amidation, addition of glycerophosphatidyl inositol), or any combination of the above. Certain PEAMT-like polypeptides comprise a sequence of 400, 425, 450, 475, 500 amino acids or fewer.

The invention also features polypeptides comprising, consisting essentially of or consisting of such polypeptides.

In another aspect, the invention features novel isolated nucleic acid molecules encoding nematode PEAMT-like polypeptides. Such isolated nucleic acid molecules include

nucleic acids comprising, consisting essentially of or consisting of the nucleotide sequence set forth in SEQ ID NO: 1, 2, 3, 4, 5 and 6 or SEQ ID NO: 13, 14, 15, 16, 17 and 18. Also included are isolated nucleic acid molecules having the same sequence as or encoding the same polypeptide as a nematode phosphoethanolamine n-methyltransferase-like gene (other than *C. elegans* PEAMT-like genes).

Also featured are: 1) isolated nucleic acid molecules having a strand that hybridizes under low stringency conditions to a single stranded probe of the sequences of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6, or their complements and, optionally, encodes polypeptides of between 400 and 500 amino acids; 2) isolated nucleic acid molecules having a strand that hybridizes under high stringency conditions to a single stranded probe of the sequence of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6 or their complements and, optionally, encodes polypeptides of between 400 and 500 amino acids; 3) isolated nucleic acid fragments of a PEAMT-like nucleic acid molecule, e.g., a fragment of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6 that is about 500, 750, 1000, 1250, 1500, 1750 or more nucleotides in length or ranges between such lengths; and 4) oligonucleotides that are complementary to a PEAMT-like nucleic acid molecule or a PEAMT-like nucleic acid complement, e.g., an oligonucleotide of about 10, 15, 18, 20, 22, 24, 28, 30, 35, 40, 50, 60, 70, 80, or more nucleotides in length. Exemplary oligonucleotides are oligonucleotides which anneal to a site located between nucleotides about 1 to 24, 1 to 48, 1 to 60, 1 to 120, 24 to 48, 24 to 60, 49 to 60, 61 to 180, 381 to 420, 421 to 480, 451 to 466, 451 to 489, 451 to 516, 500 to 1450 of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6. Nucleic acid fragments include the following non-limiting examples: nucleotides about 1 to 200, 100 to 300, 200 to 400, 300 to 500, 300 to 466, 300 to 516, 300 to 489, 489 to 1450 of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6. Also within the invention are nucleic acid molecules that hybridize under stringent conditions to nucleic acid molecule consisting of SEQ ID NO: 1, 2, 3, 4, 5 or 6 and comprise 3,000, 2,000, 1,000 or fewer nucleotides. The isolated nucleic acid can further include a heterologous promoter or other sequences required for transcription or translation of the nucleic acid molecule in a cell, e.g., a mammalian or eukaryotic or prokaryotic cell, operably linked to the PEAMT-like nucleic acid molecule. The isolated nucleic acid molecule can encode a polypeptide having PEAMT enzymatic activity. Thus, as explained in greater detail below, a polypeptide having PEAMT1 enzymatic activity can catalyze the conversion of ethanolamine to monomethylethanolamine, and a polypeptide

having PEAMT2 enzymatic catalyzes the converstion of monomethylethanolamine to dimethylethanolamine and the conversion of dimethylethanolamine to choline

A molecule featured herein can be from a nematode of the class *Araeolaimida, Ascaridida, Chromadorida, Desmodorida, Diplogasterida, Monhysterida, Mononchida, Oxyurida, Rhigonematida, Spirurida, Enoplia, Desmoscolecidae, Rhabditida*, or *Tylenchida.* Alternatively, the molecule can be from a species of the class *Rhabditida*, particularly a species other than *C. elegans* or *C. briggsae.*

In another aspect, the invention features a vector, e.g., a vector containing an aforementioned nucleic acid. The vector can further include one or more regulatory elements, e.g., a heterologous promoter or elements required for translation. The regulatory elements can be operably linked to the phosphoethanolamine n-methyltransferase-like nucleic acid molecules in order to express a PEAMT-like nucleic acid molecule. In yet another aspect, the invention features a transgenic cell or transgenic organism having in its genome a transgene containing an aforementioned PEAMT-like nucleic acid molecule and a heterologous nucleic acid, e.g., a heterologous promoter.

In still another aspect, the invention features an antibody, e.g., an antibody, antibody fragment, or derivative thereof that binds specifically to an aforementioned polypeptide. Such antibodies can be polyclonal or monoclonal antibodies. The antibodies can be modified, e.g., humanized, rearranged as a single-chain, or CDR-grafted. The antibodies may be directed against a fragment, a peptide, or a discontinuous epitope from a PEAMT-like polypeptide.

In another aspect, the invention features a method of screening for a compound that binds to a nematode PEAMT-like polypeptide, e.g., an aforementioned polypeptide. The method includes providing the nematode polypeptide; contacting a test compound to the polypeptide; and detecting binding of the test compound to the nematode polypeptide. In one embodiment, the method further includes contacting the test compound to a mammalian PEAMT-like polypeptide and detecting binding of the test compound to the mammalian PEAMT-like polypeptide. A test compound that binds the nematode PEAMT-like polypeptide with at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold affinity greater relative to its affinity for the mammalian (e.g., a human) PEAMT-like polypeptide can be identified.

The invention also features methods for identifying compounds that alter (increase or decrease) the activity of a nematode phosphoethanolamine n-methyltransferase-like polypeptide. The method includes contacting the test compound to the nematode PEAMT-like polypeptide and detecting a PEAMT-like activity. A decrease in the level of PEAMT-like activity of the polypeptide relative to the level of PEAMT-like activity of the polypeptide in the absence of the test compound is an indication that the test compound is an inhibitor of the PEAMT-like activity. In still another embodiment, the method further includes contacting a test compound such as an allosteric inhibitor or other types of inhibitors that prevent binding of the PEAMT-like polypeptide to other molecules or proteins. Such inhibitory compounds are potential selective agents for reducing the viability of a nematode expressing a PEAMT-like polypeptide, e.g., *A. suum, H. contortus, M. incognita M. javanica* and/or *S. stercoralis*. These methods can also include contacting the compound with a plant (e.g., a spinach) phosphoethanolamine n-methyltransferase polypeptide; and detecting a PEAMT-like activity. A compound that decreases nematode phosphoethanolamine n-methyltransferase activity to a greater extent than it decreases plant PEAMT-like polypeptide activity could be useful as a selective inhibitor of the nematode polypeptide. A desirable compound can exhibit 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold or greater selective activity against the nematode polypeptide.

Another featured method is a method of screening for a compound that alters (increases or decreases) an activity of a phosphoethanolamine n-methyltransferase-like polypeptide or alters binding or regulation of other polypeptides by PEAMT. The method includes providing the polypeptide; contacting a test compound to the polypeptide; and detecting an PEAMT-like activity or the activity of polypeptides bound or regulated by the PEAMT, wherein a change in activity of PEAMT-like polypeptides or other downstream polypeptides relative to the PEAMT-like activity of the polypeptide or downstream polypeptides in the absence of the test compound is an indication that the test compound alters the activity of the polypeptide(s). The method can further include contacting the test compound to a plant (e.g., a spinach) phosphoethanolamine n-methyltransferase polypeptide and measuring the methyltransferase activity of the plant PEAMT polypeptide. A test compound that alters the activity of the nematode PEAMT-like polypeptide at a given concentration and that does not substantially alter the activity of the plant methyltransferase

polypeptide or downstream polypeptides at the given concentration can be identified. An additional method includes screening for both binding to a PEAMT-like polypeptide and for an alteration in the activity of a PEAMT-like polypeptide. Yet another featured method is a method of screening for a compound that alters (increases or decreases) the viability or fitness of a transgenic cell or organism or nematode. The transgenic cell or organism has a transgene that expresses a phosphoethanolamine n-methyltransferase-like polypeptide. The method includes contacting a test compound to the transgenic cell or organism and detecting changes in the viability or fitness of the transgenic cell or organism. This alteration in viability or fitness can be measured relative to an otherwise identical cell or organism that does not harbor the transgene.

Also featured is a method of screening for a compound that alters the expression of a nematode nucleic acid encoding a phosphoethanolamine n-methyltransferase-like polypeptide, e.g., a nucleic acid encoding a *A. suum, H. contortus, M. incognita M. javanica* and/or *S. stercoralis* PEAMT-like polypeptide. The method includes contacting a cell, e.g., a nematode cell, with a test compound and detecting expression of a nematode nucleic acid encoding a PEAMT-like polypeptide, e.g., by hybridization to a probe complementary to the nematode nucleic acid encoding a PEAMT-like polypeptide or by contacting polypeptides isolated from the cell with a compound, e.g., antibody that binds a PEAMT-like polypeptide. Compounds identified by the method are also within the scope of the invention.

In yet another aspect, the invention features a method of treating a disorder (e.g., an infection) caused by a nematode, e.g., *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis,* in a subject, e.g., a host plant or host animal. The method includes administering to the subject an effective amount of an inhibitor of a PEAMT-like polypeptide activity or an inhibitor of expression of a PEAMT-like polypeptide. Non-limiting examples of such inhibitors include: an antisense nucleic acid (or PNA) to an PEAMT-like nucleic acid, an antibody to a PEAMT-like polypeptide, or a small molecule identified as a PEAMT-like polypeptide inhibitor by a method described herein.

A "purified polypeptide", as used herein, refers to a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated. The polypeptide can constitute at least 10, 20, 50 70, 80 or 95% by dry weight of the purified preparation.

An "isolated nucleic acid" is a nucleic acid, the structure of which is not identical to that of any naturally occurring nucleic acid, or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example: (a) a DNA which is part of a naturally occurring genomic DNA molecule but is not flanked by both of the nucleic acid sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of different (i) DNA molecules, (ii) transfected cells, or (iii) cell clones in a DNA library such as a cDNA or genomic DNA library. Isolated nucleic acid molecules according to the present invention further include molecules produced synthetically, as well as any nucleic acids that have been altered chemically and/or that have modified backbones.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" refers to the sequence of the nucleotides in the nucleic acid molecule, the two phrases can be used interchangeably.

The term "substantially pure" as used herein in reference to a given polypeptide means that the polypeptide is substantially free from other biological macromolecules. The substantially pure polypeptide is at least 75% (e.g., at least 80, 85, 95, or 99%) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The "percent identity" of two amino acid sequences or of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci.* USA 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90:5873-5877. Such an algorithm is incorporated into the Blastall (BLASTP, BLASTX, TBLASTN, TBLASTX) or Bl2seq programs (version 2.x and later) of Altschul et al. (1990). *J. Mol. Biol.* 215:403-10. Bl2seq performs a comparison between the subject sequence and a target sequence using either the BLASTN (used to compare nucleic acid sequences) or

BLASTP (used to compare amino acid sequences) algorithm. Typically, the default parameters of a BLOSUM62 scoring matrix, gap existence cost of 11 and extension cost of 1, a word size of 3, an expect value of 10, a per residue cost of 1 and a lambda ratio of 0.85 are used when performing amino acid sequence alignments. The output file contains aligned regions of homology between the target sequence and the subject sequence. Once aligned, a length is determined by counting the number of consecutive nucleotides or amino acid residues (*i.e.,* excluding gaps) from the target sequence that align with sequence from the subject sequence starting with any matched position and ending with any other matched position. A matched position is any position where an identical nucleotide or amino acid residue is present in both the target and subject sequence. Gaps of one or more residues can be inserted into a target or subject sequence to maximize sequence alignments between structurally conserved domains (*e.g.,* É-helices, é-sheets, and loops).

The percent identity over a particular length is determined by counting the number of matched positions over that particular length, dividing that number by the length and multiplying the resulting value by 100. For example, if (*i*) a 500 amino acid target sequence is compared to a subject amino acid sequence, (*ii*) the Bl2seq program presents 200 amino acids from the target sequence aligned with a region of the subject sequence where the first and last amino acids of that 200 amino acid region are matches, and (*iii*) the number of matches over those 200 aligned amino acids is 180, then the 500 amino acid target sequence contains a length of 200 and a sequence identity over that length of 90% (*i.e.,* 180 ÷ 200 x 100 = 90).

It will be appreciated that a nucleic acid or amino acid target sequence that aligns with a subject sequence can result in many different lengths with each length having its own percent identity. It is noted that the percent identity value can be rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It is also noted that the length value will always be an integer.

The identification of conserved regions in a template, or subject, polypeptide can facilitate homologous polypeptide sequence analysis. Conserved regions can be identified by locating a region within the primary amino acid sequence of a template polypeptide that is a repeated sequence, forms some secondary structure (e.g., helices and beta sheets), establishes

positively or negatively charged domains, or represents a protein motif or domain. See, e.g., the Pfam web site describing consensus sequences for a variety of protein motifs and domains on the Internet at sanger.ac.uk/Pfam/ and genome.wustl.edu/Pfam/. A description of the information included at the Pfam database is described in Sonnhammer et al. (1998) *Nucl. Acids Res.* 26: 320-322; Sonnhammer et al. (1997) *Proteins* 28:405-420; and Bateman et al. (1999) *Nucl. Acids Res.* 27:260-262. From the Pfam database, consensus sequences of protein motifs and domains can be aligned with the template polypeptide sequence to determine conserved region(s).

As used herein, the term "transgene" means a nucleic acid sequence (encoding, e.g., one or more subject polypeptides), which is partly or entirely heterologous, i.e., foreign, to the transgenic plant, animal, or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic plant, animal, or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the plant's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and other nucleic acid sequences, such as introns, that may be necessary for optimal expression of the selected nucleic acid, all operably linked to the selected nucleic acid, and may include an enhancer sequence.

As used herein, the term "transgenic cell" refers to a cell containing a transgene.

As used herein, a "transgenic plant" is any plant in which one or more, or all, of the cells of the plant includes a transgene. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by T-DNA mediated transfer, electroporation, or protoplast transformation. The transgene may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

As used herein, the term "tissue-specific promoter" means a DNA sequence that serves as a promoter, i.e., regulates expression of a selected DNA sequence operably linked to the promoter, and which affects expression of the selected DNA sequence in specific cells of a tissue, such as a leaf, root, or stem.

As used herein, the terms "hybridizes under stringent conditions" and "hybridizes under high stringency conditions" refer to conditions for hybridization in 6X sodium

chloride/sodium citrate (SSC) buffer at about 45 °C, followed by two washes in 0.2 X SSC buffer, 0.1% SDS at 60 °C or 65 °C. As used herein, the term "hybridizes under low stringency conditions" refers to conditions for hybridization in 6X SSC buffer at about 45 °C, followed by two washes in 6X SSC buffer, 0.1% (w/v) SDS at 50 °C.

A "heterologous promoter", when operably linked to a nucleic acid sequence, refers to a promoter which is not naturally associated with the nucleic acid sequence.

As used herein, an agent with "anthelminthic or anthelmintic or antihelminthic activity" is an agent, which when tested, has measurable nematode-killing activity or results in infertility or sterility in the nematodes such that unviable or no offspring result. In the assay, the agent is combined with nematodes, e.g., in a well of microtiter dish having agar media or in the soil containing the agent. Staged adult nematodes are placed on the media. The time of survival, viability of offspring, and/or the movement of the nematodes are measured. An agent with "anthelminthic or anthelmintic or antihelminthic activity" reduces the survival time of adult nematodes relative to unexposed similarly staged adults, e.g., by about 20%, 40%, 60%, 80%, or more. In the alternative, an agent with "anthelminthic or anthelmintic or antihelminthic activity" may also cause the nematodes to cease replicating, regenerating, and/or producing viable progeny, e.g., by about 20%, 40%, 60%, 80%, or more.

As used herein, the term "binding" refers to the ability of a first compound and a second compound that are not covalently linked to physically interact. The apparent dissociation constant for a binding event can be 1 mM or less, for example, 10 nM, 1 nM, 0.1 nM or less.

As used herein, the term "binds specifically" refers to the ability of an antibody to discriminate between a target ligand and a non-target ligand such that the antibody binds to the target ligand and not to the non-target ligand when simultaneously exposed to both the given ligand and non-target ligand, and when the target ligand and the non-target ligand are both present in molar excess over the antibody.

As used herein, the term "altering an activity" refers to a change in level, either an increase or a decrease in the activity, (e.g., an increase or decrease in the ability of the polypeptide to bind or regulate other polypeptides or molecules) particularly a PEAMT-like or PEAMT activity. The change can be detected in a qualitative or quantitative observation. If a quantitative observation is made, and if a comprehensive analysis is performed over a

plurality of observations, one skilled in the art can apply routine statistical analysis to identify modulations where a level is changed and where the statistical parameter, the *p* value, is less than 0.05.

In part, the nematode phosphoethanolamine n-methyltransferase proteins and nucleic acids described herein are novel targets for anti-nematode vaccines, pesticides, and drugs. Inhibition of these molecules can provide means of inhibiting nematode metabolism and/or the nematode life-cycle.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 depicts the cDNA sequence of *A. summ* phosphoethanolamine n-methyltransferase (PEAMT1) (SEQ ID NO: 1), its corresponding encoded amino acid sequence (SEQ ID NO: 7), and its open reading frame (SEQ ID NO: 13).

FIG. 2 depicts the cDNA sequence of *H. contortus* phosphoethanolamine n-methyltransferase (PEAMT1) (SEQ ID NO: 2), its corresponding encoded amino acid sequence (SEQ ID NO: 8), and its open reading frame (SEQ ID NO: 14).

FIG. 3 depicts the cDNA sequence of *M. incognita* phosphoethanolamine n-methyltransferase (PEAMT1) (SEQ ID NO: 3), its corresponding encoded amino acid sequence (SEQ ID NO: 9), and its open reading frame (SEQ ID NO: 15).

FIG. 4 depicts the cDNA sequence of *S. stercoralis* phosphoethanolamine n-methyltransferase (PEAMT1) (SEQ ID NO: 4), its corresponding encoded amino acid sequence (SEQ ID NO: 10), and its open reading frame (SEQ ID NO: 16).

FIG. 5 depicts the cDNA sequence of *A. summ* phosphoethanolamine n-methyltransferase (PEAMT2) (SEQ ID NO: 5), its corresponding encoded amino acid sequence (SEQ ID NO: 11), and its open reading frame (SEQ ID NO: 17).

FIG. 6 depicts the cDNA sequence of *M. javanica* phosphoethanolamine n-methyltransferase (PEAMT2) (SEQ ID NO: 6), its corresponding encoded amino acid sequence (SEQ ID NO: 12), and its open reading frame (SEQ ID NO: 18).

FIG. 7 is an alignment of the sequences of *A. summ, H. contortus, M. incognita* and *S. stercoralis* phosphoethanolamine n-methyltransferase-like polypeptides (SEQ ID NO: 7, 8, 9 and 10) and *C. elegans* PEAMT1-like polypeptides (SEQ ID NO: 19 and 20).

FIG. 8 is an alignment of the sequences of *A. summ* and *M. javanica* phosphoethanolamine n-methyltransferase-like polypeptides (SEQ ID NO: 11 and 12) and *C. elegans* PEAMT2-like polypeptide (SEQ ID NO: 21).

DETAILED DESCRIPTION

Choline (Cho) plays a number of important roles in biological systems. In bacteria, fungi, plants and animals, phosphatidylcholine is a major component of membrane phospholipids and the free base is a precursor to the neurotransmitter acetylcholine in animals. Choline is also an intermediate in glycine betaine (a compound that increases tolerance to osmotic stresses) synthesis in plants (McNeil et al. (2001) *Proc Natl Acad Sci USA* 98:10001-5). Choline is an essential nutrient in humans and other animals, and also plays a critical role in brain development in humans (Sheard et al. (1986) *Am J Clin Nutr.* 1986 43:219-24; Tayek et al. (1990) *J Am Coll Nut* 9:76-83). Most organisms can incorporate choline into phosphatidylcholine using a pathway that transfers a choline moiety from CDP-choline to diacylglycerol. In similar fashion, choline precursors such as ethanolamine (EA), monomethylethanolamine (MME) and dimethylethanolamine (DME) can also be incorporated into phospholipids via the CPD-choline or Kennedy pathway. Rhizobacteria have an additional Kennedy-independent pathway that also allows the incorporation of choline excreted from plant roots directly into phospholipids (Rudder et al. (1999) *J Biol Chem.* 274:20011-6; Lopez-Lara & Geiger (2001) *J Biotechnol* 91:211-21).

Among those organisms that can synthesize choline, different biosynthetic pathways are used to make choline from ethanolamine via the successive addition of methyl groups using S-adenosyl methionine (SAM) as the methyl donor. These pathways differ in whether they use the free base (ethanolamine), the phosphobase (phosphoethanolamine), or the phosphatidyl base (phosphatidylethanolamine) as the methylation substrate. Plants are unusual in that they can methylate the free base, phosphobase or phosphatidylbase (phospholipid substrate) (Bolognese & McGraw (2000) *Plant Physiol.* 124(4):1800-13; Nuccio et al. (2000) *J Biol Chem* 275(19):14095-101; Charron et al. (2002). *Plant Physiol.*

129(1):363-73). However, the conversion of phosphatidylethanolamine to phosphatidylmonomethylethanolamine has not been demonstrated in plants, so the first methylation reaction must occur at either the free base or the phosphobase level. It is now thought that in many plants the major flux occurs at the phosphobase level, catalyzed by the phosphoethanolamine *N*-methyltransferase enzyme (PEAMT) (i.e., pEA ⇒ pMME).

In contrast, in most other organisms, methylation is carried out primarily at the phospholipid level. The complete reaction (i.e., Ptd-EA ⇒ Ptd-MME ⇒ Ptd-DME ⇒ PtdCho) requires a single enzyme in bacteria and mammals and two separate enzymes in fungi (Kanipes & Henry. (1997) *Biochim Biophys Acta.* 1348(1-2):134-41; Vance et al. (1997) *Biochim Biophys Acta.* 1348(1-2):142-50; Hanada et al. (2001) *Biosci Biotechnol Biochem.* 65(12):2741-8). Mammalian nerve cells are reported to have additional phospho-base methylation activity and three distinct enzymes appear to be involved (Andriamampandry et al. (1992) *Biochem J.* 288 (1):267-72; Mukherjee et al. (1995) *Neurochem Res.* 20(10):1233-7).

Plant methyltransferases from spinach and *Arabidopsis* have been cloned by complementation of choline biosynthetic mutants in fission and budding yeast, respectively (Bolognese & McGraw (2000) *Plant Physiol.* 124(4):1800-13; Nuccio et al. (2000) *J Biol Chem.* 275(19):14095-101). In contrast to yeast methyltransferases, which act on the phosphatidylethanolamine, these plant enzymes have been shown to act on phosphoethanolamine. A similar gene has recently been cloned from chilled wheat tissues (Charron et al. (2002). *Plant Physiol.* 129(1):363-73). The plant enzymes are predicted to encode soluble proteins of approximately 55kDa that have two domains containing separate SAM binding sites. Each domain contains motifs - termed I, post-I, II, and III – that are conserved among SAM-dependent methyltransferases. cDNA clones encompassing partial sequence from both SAM binding sites have been isolated from numerous plants, including *Oryza sativa, Brassica napus, Gossypium hirsutum,* and *Hordeum vulgare.* The plant methyltransferase structure is thought to have arisen from a gene duplication event, since prokaryotic and animal methyltransferases are approximately half the size of the plant enzymes and have only one methyltransferase domain.

Some basic kinetic characteristics of the spinach methyltransferase have been determined from enzyme preparations isolated from fission yeast overexpressing it. Enzyme

activity is dependent on SAM and phosphoethanolamine concentrations. In the presence of these substrates, methyltransferase-containing extracts catalyze the formation of monomethyl- and dimethylphosphoethanolamine as well as phosphocholine. The appearance of these intermediates suggests that they are precursors to phosphocholine. A truncated version of the spinach enzyme lacking the second SAM binding site can accomplish the first methylation converting phosphoethanolamine to monomethylphosphoethanolamine, but cannot perform the second and third methylation steps. It is presumed that the C-terminal half can carry out the second and third methylation reactions.

The *C. elegans* genome contains two PEAMT-like genes and several homologs are found in other nematode EST datasets suggesting that these genes are widely distributed in *Nematoda*. The nematode proteins and plant homologs are all presumably localized in the cytosol as in the case of the wheat PEAMT as they lack secretion leaders (analyzed by methods available on the Internet at cbs.dtu.dk/services/TargetP)or transmembrane regions (analyzed by available on the Internet at cbs.dtu.dk/services/TNHMM).

One of the *C. elegans* PEAMT genes (PEAMT2) encodes a polypeptide which is 437 amino acids long (accession number AAB04824.1, wormbase locus F54D11.1) and shows significant similarity to the C-terminal half of the spinach phosphoethanolamine n-methyltransferase and other plant homologs with two SAM binding domains. The second *C. elegans* PEAMT gene appears to encode at least to two different splice variants (PEAMT1a and PEAMT1b). PEAMT1a and b are 495 and 484 amino acids long, respectively (accession number AAA81102.1, wormbase locus ZK622.3a and ZK622.3b) and are most similar to the N-terminal half of the plant PEAMTs. A PFAM analysis (available on the Internet at pfam.wustl.edu) supports the blast predictions that whereas the plant PEAMTs contain two canonical methyltransferase domains, the nematode proteins contain an N-terminal MT domain in PEAMT1 and a C-terminal MT domain in PEAMT2. PEAMT1 and PEAMT2 have 30-40% amino acid identity to their plant homologs in the regions that align. The similarity between PEAMT1 and PEAMT2 is low (22 % amino acid identity) and is restricted to a small 127 amino acid region in their C-terminal domains.

Given the similarity of PEAMT1 and PEAMT2 to the N- and C-terminal domains of the plant phosphoethanolamine *N*-methyltransferases (e.g. spinach and *Arabidopsis*) respectively, their similar larval lethal RNAi phenotypes and the observation that the N-

terminal half of the spinach enzyme is only capable of the first methylation reaction, we predicted that PEAMT1 would catalyze the conversion of pEA to pMME (the first methylation) and PEAMT2 would catalyze the conversion of pMME to pDME and pDME to pCHO. This hypothesis was confirmed by chemical complementation of the *C. elegans* PEAMT1 or PEAMT2 RNAi phenotypes with EA, MME, DME or Cho (see Table 1). As predicted, the PEAMT1 larval lethal RNAi phenotype is suppressed by MME, DME and Cho but not by EA whereas the PEAMT2 RNAi is rescued only by Cho and not by MME, DME, or EA singly or in combination.

This invention describes a novel class of nematode genes related to *C. elegans* proteins ZK622.3a (gi|28275069|gb|AAO38583.1|[28275069]), ZK622.3b (gi|15487647|gb|AAL00881.1|U39998_4[15487647]) and F54D11.1 (gi|1458245|gb|AAB04824.1|[1458245]). The nematode genes can be shown by a BLAST bioinformatics analysis and phylogenetic tree building to be related to the plant phosphoethanolamine n-methyltransferase gene family. This gene family appears to be wide spread in plants and nematodes but not in arthropods, vertebrates, fungi or bacteria. We have identified additional homologs in the nematodes *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis.* Importantly, we have shown that these proteins are essential for the viability of *C. elegans* using RNAi, which together with the redundancy of choline synthesis in plants and absence of clear homologs in vertebrates suggests that these proteins are promising targets for anti-parasitic compounds.

The nematode proteins and plant homologs are all presumably localized in the cytosol as in the case of the wheat PEAMT as they lack secretion leaders (analyzed by methods available on the Internet at cbs.dtu.dk/services/Target P) (http://www.cbs.dtu.dk/services/TargetP/) or transmembrane regions (analyzed by available on the Internet at cbs.dtu.dk/services.TMHMM).

The present invention provides nucleic acid sequences from nematodes encoding phosphoethanolamine n-methyltransferase-like polypeptides. The *A. suum* nucleic acid molecule (SEQ ID NO: 1) and the encoded PEAMT1-like polypeptide (SEQ ID NO: 7) are depicted in FIG. 1. The *H. contortus* nucleic acid molecule (SEQ ID NO: 2) and the PEAMT1-like polypeptide (SEQ ID NO: 8) are depicted in FIG. 2. The *M. incognita* nucleic acid molecule (SEQ ID NO: 3) and the encoded PEAMT1-like polypeptide (SEQ ID NO: 9)

are depicted in FIG. 3. The *S. stercoralis* nucleic acid molecule (SEQ ID NO: 4) and the PEAMT1-like polypeptide (SEQ ID NO: 10) are depicted in FIG. 4. The *A. suum* nucleic acid molecule (SEQ ID NO: 5) and the encoded PEAMT2-like polypeptide (SEQ ID NO: 11) are depicted in FIG. 5. The *M. javanica* nucleic acid molecule (SEQ ID NO: 6) and the PEAMT2-like polypeptide (SEQ ID NO: 12) are depicted in FIG. 6. Certain sequence information for the PEAMT-like genes described herein is summarized in Table 1, below.

Table 1

Species	CDNA	ORF	Polypeptide	Figure
A. suum	SEQ ID NO: 1	SEQ ID NO: 7	SEQ ID NO: 13	Fig. 1
H. contortus	SEQ ID NO: 2	SEQ ID NO: 8	SEQ ID NO: 14	Fig. 2
M. incognita	SEQ ID NO: 3	SEQ ID NO: 9	SEQ ID NO: 15	Fig. 3
S. stercoralis	SEQ ID NO: 4	SEQ ID NO: 10	SEQ ID NO: 16	Fig. 4
A. suum	SEQ ID NO: 5	SEQ ID NO: 11	SEQ ID NO: 17	Fig. 5
M. javanica	SEQ ID NO: 6	SEQ ID NO: 12	SEQ ID NO: 18	Fig. 6

The invention is based, in part, on the discovery of PEAMT-like sequences from *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis*. The following examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All of the publications cited herein are hereby incorporated by reference in their entirety.

EXAMPLES

A TBLASTN query with the *C. elegans* genes ZK622.3a (gi|1055130|gb|AAA81102.1|[1055130]) and F54D11.1 (gi|1458245|gb|AAB04824.1|[1458245]) identified multiple expressed sequence tags (ESTs are short nucleic acid fragment sequences from single sequencing reads) in dbest that are predicted to encode a portion of PEAMT-like enzymes in multiple nematode species.

PEAMT1-like ESTs identified as similar to *C. elegans* AAA81102.1 included *Ancylostoma caninum* (GenBank® Identification No: 15766091), *Ascaris suum* (GenBank® Identification No: 17993264), *Strongyloides stercoralis* (GenBank® Identification No:

12714760), *Haemonchus contortus* (GenBank® Identification No: 27590930), multiple from *Pristionchus pacificus* (GenBank® Identification Nos: 6067811, 15339937, 6081336, 5816211), and *Meloidogyne incognita* (GenBank® Identification No: 21652426), all from McCarter, et al. (1999) Washington University Nematode EST Project.

PEAMT2-like ESTs identified as similar to *C. elegans* AAB04824.1 included *Meloidogyne javanica* (GenBank® Identification No: 14624708); *Meloidogyne incognita* (GenBank® Identification No: 9033918); *Globodera rostochiensis* (GenBank® Identification No: 18080101); and multiple from *Ascaris suum* (GenBank® Identification Nos: 15498087, 17991691, 18688588, 17992674, 18688567, 18054078, 18828817, 18688268, 18053654, 17992401, 17991763, 17992578, 18689591, 18688755, 18688890, 18686360, 17993455,17992123).

Full-length Phosphoethanolamine n-Methyltransferase1-like cDNA sequences

Plasmid clone, Div2728, corresponding to the *S. stercoralis* EST sequence (GenBank® Identification No: 12714760) was obtained from the Genome Sequencing Center (St. Louis, MO). The cDNA insert in the plasmid was sequenced in its entirety. Unless otherwise indicated, all nucleotide sequences determined herein were sequenced with an automated DNA sequencer (such as model 373 from Applied Biosystems, Inc.) using processes well-known to those skilled in the art. Primers used are listed in Table 2 (see below). Full sequence data for the *S. stercoralis* PEAMT1 was obtained from Div2728, including nucleotide sequence for codons 1-469 and additional 5' and 3' untranslated sequences.

Table 2

Name	Sequence	SEQ ID NO:	Homology to
T7	gtaatacgactcactatagggc	22	vector polylinker primer
T3	aattaaccctcactaaaggg	23	vector polylinker primer
SP6	gatttaggtgacactatag	24	vector polylinker primer
MTas-1	atgcctgcggcagagcg	25	*As* PEAMT1 (codons 71-76)
AUAP	ggccacgcgtcgactagtac	26	abridged universal primer
SL1	gggtttaattacccaagtttga	27	nematode transpliced leader
Oligo dT	ggccacgcgtcgactagtacttttttttttttttt	28	universal primer to poly A tail

MU19-A	atggtgaacgttcgtcgtgc	29	*Ce* PEAMT1$_a$ (genomic)
MU19-B	catacgtatttctcatcatc	30	*Ce* PEAMT1$_a$ (genomic)
MU21-A	ccagattattaccaacgccg	31	*Ce* PEAMT2 (genomic)
MU21-B	tgaacttacatagattcttg	32	*Ce* PEAMT2 (genomic)
MTmi-9	gcaattgaatatatgcggatg	33	*Mi* PEAMT1 (codons192-197)
MTmi-8	ctatccgaattggaatgtagcg	34	*Mi* PEAMT1 (codons 176-181)
MTmi-4	cattccaattcggatagtatc	35	*Mi* PEAMT1 (codons 177-183)
GeneRacer	cgactggagcacgaggacactga	36	GeneRacer kit component
GeneRacer ne	ggacactgacatggactgaaggagta	37	GeneRacer kit component
MThc-1	caacggatttcatcgaatcg	38	*Hc* PEAMT1 (codons 79-84)
MThc-4	ccacgtctttgttggttagg	39	*Hc* PEAMT1 (codons 50-55)
RNA oligo	cgacuggagcacgaggacacugacauggacugaaggagu	40	GeneRacer kit component
SL2	ggttttaacccagtatctcaag	41	Haemonchus transpliced leader
Met12	gcatcagcaatttgatattc	42	*Mj* PEAMT2 (codons 302-308)
Met28	ccgcaatatccagaagac	43	*As* PEAMT2 (codons 159-164)
Met39	cagatctcgatacattcg	44	*As* PEAMT2 (codons 67-73)
D2728-seqF1	gttctgaaccatcaacaag	45	*Ss* PEAMT1 (codons 161-165)
D2728-seqR1	gctgaagttaatgaacatc	46	*Ss* PEAMT1 (codons 342-346)

Plasmid clone, Div3020, corresponding to the *A. suum* EST sequence (GenBank® Identification No: 17993264) was obtained from the Genome Sequencing Center (St. Louis, MO). The cDNA insert in the plasmid was sequenced in its entirety. Partial sequence data for the *A. suum* PEAMT1 was obtained from Div3020, including nucleotide sequence for codons 1-88 and additional 5' untranslated sequence. The available sequence lacked the last 372 codons of the *A. suum* PEAMT1, as well as 3' untranslated sequence.

To obtain the missing 3' sequence of the *A. suum* PEAMT1 gene, the 3' RACE technique was applied. First strand cDNA synthesis from total *A. suum* RNA was performed using an oligo dT primer (SEQ ID NO: 28). The cDNA was then directly PCR amplified using a gene specific primer (MTas-1; SEQ ID NO: 25) designed from the known sequence that anneals within the cDNA molecule of interest, and the AUAP primer, which is homologous to the 3' end of all cDNAs amplified with the oligo dT primer (SEQ ID NO: 28). This procedure was performed to generate clone Div3465, which contains codons 71-460 in

addition to 3' untranslated sequences. Taken together, clones Div3020 and Div3465 contain sequences comprising the complete open reading frame of PEAMT1 gene of *A. suum*.

Plasmid clone, Div3440, corresponding to the *M. incognita* EST sequence (GenBank® Identification No: 21652426) was obtained from the Genome Sequencing Center (St. Louis, MO). The cDNA insert in the plasmid was sequenced in its entirety. Partial sequence data for the *M. incognita* PEAMT1 was obtained from Div3440, including nucleotide sequence for codons 14-227. The available sequence lacked the first 13 codons and the last 366 codons, as well as both the 5' and 3' untranslated sequences.

To obtain the missing 3' end of the *M. incognita* PEAMT1 gene, the 3' RACE technique was applied. First strand cDNA synthesis from total *M. incognita* RNA was performed using an oligo dT primer (SEQ ID NO: 28). The cDNA was then directly PCR amplified using a gene specific primer (MTmi-4; SEQ ID NO: 35) designed from the known sequence that anneals within the cDNA molecule of interest, and the AUAP primer, which is homologous to the 3' end of all cDNAs amplified with the oligo dT primer (SEQ ID NO: 28). This procedure was performed to generate clone Div3640, which contains codons 177-457 in addition to 3' untranslated sequences.

To obtain the missing 5' sequence of the *M. incognita* PEAMT1 gene, the 5' oligo-capped RACE method (GeneRacer™ kit from Invitrogen Life Technologies) was applied. This technique results in the selective ligation of an RNA oligonucleotide (SEQ ID NO: 40) to the 5' ends of decapped mRNA using T4 RNA ligase. First strand cDNA synthesis from total *M. incognita* oligo-capped RNA was performed using an internal gene specific primer (MTmi-9; SEQ ID NO: 33) designed from the known sequence that anneals within the cDNA molecule of interest. The first strand cDNA was then directly PCR amplified using a nested gene specific primer (MTmi-8; SEQ ID NO: 34) designed from known sequence that anneals within the cDNA molecule of interest, and the GeneRacer™5' nested oligo (SEQ ID NO: 37), which is homologous to the 5' end of all cDNAs amplified with the GeneRacer™ oligo-capped RNA method. This procedure was performed to generate clone Div3845, which c0ontains codons 1-13 in addition to 5' untranslated sequences. The missing nucleotide sequence encoding codons 92-176 for the *M. incognita* PEAMT1 gene was obtained by the 5' oligo capped RACE method (GeneRacer™ kit from Invitrogen Life Technologies) as described previously. First strand cDNA synthesis from total *M. incognita* oligo-capped

RNA was performed using an internal gene specific primer (MTmi-9; SEQ ID NO: 33) designed from the known sequence that anneals within the cDNA molecule of interest. The first strand cDNA was then directly PCR amplified using a nested gene specific primer (MTmi-8; SEQ ID NO: 34) designed from known sequence that anneals within the cDNA molecule of interest, and the GeneRacer™5' nested oligo (SEQ ID NO: 37), which is homologous to the 5' end of all cDNAs amplified with the GeneRacer™ oligo-capped RNA method. This procedure was performed to generate clone Div3846, which contains codons 1-181. Taken together, clones Div3440, Div3845, Div3846, and Div3640 contain sequences comprising the complete open reading frame of PEAMT1 gene of *M. incognita.*

Partial sequence data for the *H. contortus* PEAMT1 was obtained from the *H. contortus* EST (GenBank® Identification No: 27590930), including nucleotide sequence for codons 3-86. The available sequence lacked the first 2 codons and the last 374 codons of the *H. contortus* PEAMT1, as well as the 5' and 3' untranslated regions.

To obtain the 5' sequence of the *H. contortus* PEAMT1 gene, the 5' RACE technique was applied, and SL2 PCR was performed using first strand cDNA from *H. contortus* as a template (cDNA synthesis explained above). The first strand cDNA was directly PCR amplified using a gene specific primer (MThc-4; SEQ ID NO: 39) designed from known EST sequence that anneals to a site located within the cDNA of interest, and the SL2 primer (SEQ ID NO: 41), which is homologous to the 5' end of many *H. contortus* cDNAs. Amplified PCR products were then cloned into a suitable vector for DNA sequence analysis. This procedure was performed to obtain clone Div3676. This clone contains codons 1-55 in addition to 5' untranslated sequences. To obtain the 3' sequence of the *H. contortus* PEAMT1 gene, the 3' RACE technique was applied. First strand cDNA synthesis from *H. contortus* RNA was performed as described previously. The first strand cDNA was directly PCR amplified using a gene specific primer (MThc-1; SEQ ID NO: 38) designed from the known sequence that anneals within the first strand cDNA molecule of interest, and the AUAP primer (SEQ ID NO: 26), which is homologous to the 3' end of the cDNA molecule of interest. This procedure was performed to generate clone Div3650, which contains codons 79-460 in addition to 3' untranslated sequences. Taken together, clones Div3650, Div3676, and the known EST sequence contain sequences comprising the complete open reading frame of the PEAMT1 gene of *H. contortus.*

Full-length Phosphoethanolamine n-Methyltransferase2-like cDNA sequences

Plasmid clone, Div2562, corresponding to the *M. javanica* EST sequence (GenBank® Identification No: 14624708) was obtained from the Genome Sequencing Center (St. Louis, MO). The cDNA insert in the plasmid was sequenced in its entirety. Unless otherwise indicated, all nucleotide sequences determined herein were sequenced with an automated DNA sequencer (such as model 373 from Applied Biosystems, Inc.) using processes well-known to those skilled in the art. Primers used for sequencing are listed in Table 2 (see below). Partial sequence data for the *M. javanica* PEAMT2 was obtained from Div2562, including nucleotide sequence for codons 285-472 and additional 3' untranslated sequence. The clone lacked the first 284 codons of the *M. javanica* PEAMT2, as well as the 5' untranslated region.

To obtain the missing 5' sequence of the *M. javanica* PEAMT2 gene, the 5' RACE technique was applied, and SL1 PCR was performed using first strand cDNA from *M. javanica* as a template. Briefly, SL1 PCR utilizes the observation, that unlike most eukaryotic mRNAs, many nematode mRNA molecules contain a common leader sequence (5' gggtttaattacccaagtttga 3') (SEQ ID NO: 27) transpliced to their 5' ends. If this sequence is present on the 5' end of the cDNA, that cDNA can be amplified using PCR with a primer that binds to the SL1 transpliced leader and a gene-specific primer near the 3' end of the cDNA.

Briefly, following the instructions provided by Life Technologies cDNA synthesis kit, first strand cDNA synthesis was performed on total nematode RNA using SuperScript™ II Reverse Transcriptase and an oligo-dT primer (which anneals to the natural poly A tail found on the 3' end of all eukaryotic mRNA). RNase H was then used to degrade the original mRNA template. Following degradation of the original mRNA template, the first strand cDNA was directly PCR amplified without further purification using Taq DNA polymerase, a gene specific primer (Met12, SEQ ID NO: 42) designed from known sequence to the cDNA of interest, and the SL1 primer (SEQ ID NO: 27), which is homologous to the 5' end of many nematode cDNAs. Amplified PCR products were then cloned into a suitable vector for DNA sequence analysis. This procedure was performed to obtain clone Div2474. Div2474 contains codons 1-308 in addition to 5' untranslated sequences. Taken together, clones Div2562 and Div2474 contain sequences comprising the complete open reading frame of the PEAMT2 gene from *M. javanica*.

Partial sequence data for the *A. suum* PEAMT2 was obtained from the *A. suum* EST (GenBank® Identification No: 15498087), including nucleotide sequence for codons 35-205. The available sequence lacked the first 34 codons and the last 232 codons of the *A. suum* PEAMT2, as well as the 5' and 3' untranslated regions.

To obtain the 5' sequence of the *A. suum* PEAMT2 gene, the 5' RACE technique was applied, and SL1 PCR was performed using first strand cDNA from *A. suum* as a template (cDNA synthesis explained above). The first strand cDNA was directly PCR amplified using a gene specific primer (Met28; SEQ ID NO: 43) designed from the known EST sequence that anneals to a site located within the cDNA of interest, and the SL1 primer (SEQ ID NO: 27), which is homologous to the 5' end of many nematode cDNAs. Amplified PCR products were then cloned into a suitable vector for DNA sequence analysis. This procedure was performed to obtain clone Div2715. This clone contained codons 1-164 in addition to 5' untranslated sequences. To obtain the 3' sequence of the *A. suum* PEAMT2 gene, the 3' RACE technique was applied. First strand cDNA synthesis from *A. suum* RNA was performed as described previously. The first strand cDNA was directly PCR amplified using a gene specific primer (Met39; SEQ ID NO: 44) designed from the known sequence that anneals within the first strand cDNA molecule of interest, and the AUAP primer (SEQ ID NO: 26), which is homologous to the 3' end of the cDNA of interest. This procedure was performed to generate clone Div2877, which contains codons 67-437 in addition to 3' untranslated sequences. Taken together, clones Div2715 and Div2877 contain sequences comprising the complete open reading frame of PEAMT2 gene of *A. suum*.

Characterization of six nematode Phosphoethanolamine n-Methyltransferases

The sequences of the four PEAMT1-like phosphoethanolamine n-methyltransferase-like nucleic acid molecules (*A. suum, H. contortus, M. incognita* and *S. stercoralis*) and two PEAMT2-like phosphoethanolamine n-methyltransferase-like nucleic acid molecules (*A. suum* and *M. javanica*) are depicted in FIG. 1, FIG. 2, FIG. 3, FIG. 4, FIG. 5 and FIG. 6 as SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6. SEQ ID NO: 13 (*A. suum*) contains an open reading frame encoding a 460 amino acid polypeptide, SEQ ID NO: 14 (*H. contortus*) contains an open reading frame encoding a 460 amino acid polypeptide, SEQ ID NO: 15 (*M. incognita*) contains an open reading frame

encoding a 457 amino acid polypeptide, SEQ ID NO: 16 (*S. stercoralis*) contains an open reading frame encoding a 469 amino acid polypeptide, SEQ ID NO: 17 (*A. suum*) contains an open reading frame encoding a 437 amino acid polypeptide and SEQ ID NO: 18 (*M. javanica*) contains an open reading frame encoding a 472 amino acid polypeptide.

The sequence of the *A. suum* PEAMT1-like nucleic acid molecule is recited in FIG. 1 as SEQ ID NO: 1. This nucleotide sequence contains an open reading frame encoding a 460 amino acid polypeptide. The *A. suum* PEAMT1-like -protein (depicted in FIG. 1 as SEQ ID NO 7) is approximately 52% identical (in the region of shared homology) to the *C. elegans* PEAMT1-like proteins (depicted in FIG. 7 as SEQ ID NO: 19 and 20). The similarity between the PEAMT1 proteins from *A. suum* and from *C. elegans* is presented as a multiple alignment generated by the Clustal X multiple alignment program.

The sequence of the *H. contortus* PEAMT1-like nucleic acid molecule is recited in FIG. 2 as SEQ ID NO: 2. This nucleotide sequence contains an open reading frame encoding a 460 amino acid polypeptide. The *H. contortus* PEAMT1-like protein (depicted in FIG. 2 as SEQ ID NO 8) is approximately 63% identical (in the region of shared homology) to the *C. elegans* PEAMT1-like proteins (depicted in FIG. 7 as SEQ ID NO: 19 and 20). The similarity between the PEAMT1 proteins from *H. contortus* and from *C. elegans* is presented as a multiple alignment generated by the Clustal X multiple alignment program.

The sequence of the *M. incognita* PEAMT1-like nucleic acid molecule is recited in FIG. 3 as SEQ ID NO: 3. This nucleotide sequence contains an open reading frame encoding a 457 amino acid polypeptide. The *M. incognita* PEAMT1-like protein (depicted in FIG. 3 as SEQ ID NO 9) is approximately 43% identical (in the region of shared homology) to the *C. elegans* PEAMT1-like proteins (depicted in FIG. 7 as SEQ ID NO: 19 and 20). The similarity between the PEAMT1 proteins from *M. incognita* and from *C. elegans* is presented as a multiple alignment generated by the Clustal X multiple alignment program.

The sequence of the *S. stercoralis* PEAMT1-like nucleic acid molecule is recited in FIG. 4 as SEQ ID NO: 4. This nucleotide sequence contains an open reading frame encoding a 469 amino acid polypeptide. The *S. stercoralis* PEAMT1-like protein (depicted in FIG. 4 as SEQ ID NO: 10) is approximately 36% identical (in the region of shared homology) to the *C. elegans* PEAMT1-like proteins (depicted in FIG. 7 as SEQ ID NO: 19 and 20). The

similarity between the PEAMT1 proteins from *S. stercoralis* and from *C. elegans* is presented as a multiple alignment generated by the ClustalX multiple alignment program.

The sequence of the *A. suum* PEAMT2-like nucleic acid molecule is recited in FIG. 5 as SEQ ID NO: 5. This nucleotide sequence contains an open reading frame encoding a 437 amino acid polypeptide. The *A. suum* PEAMT2-like protein (depicted in FIG. 5 as SEQ ID NO 11) is approximately 48% identical (in the region of shared homology) to the *C. elegans* PEAMT2-like proteins (depicted in FIG. 8 as SEQ ID NO: 21). The similarity between the PEAMT2 proteins from *A. suum* and from *C. elegans* is presented as a multiple alignment generated by the Clustal X multiple alignment program.

The sequence of the *M. javanica* PEAMT2-like nucleic acid molecule is recited in FIG. 6 as SEQ ID NO: 6. This nucleotide sequence contains an open reading frame encoding a 472 amino acid polypeptide. The *M. javanica* PEAMT2-likeprotein (depicted in FIG. 6 as SEQ ID NO 12) is approximately 50% identical (in the region of shared homology) to the *C. elegans* PEAMT2-like proteins (depicted in FIG. 8 as SEQ ID NO: 21). The similarity between the PEAMT2 proteins from *M. javanica* and from *C. elegans* is presented as a multiple alignment generated by the Clustal X multiple alignment program.

The similarity among the *A. suum, H. contortus, M. incognita, S. stercoralis,* and *C. elegans* PEAMT1-like polypeptides is presented as a multiple alignment generated by the Clustal X multiple alignment program (depicted in FIG. 7). The similarity among the *A. suum, M. javanica* and *C. elegans* PEAMT2-like polypeptides is presented as multiple alignment generated by the Clustal X multiple alignment program (depicted in FIG. 8)

S-adenosylmethionine (SAM)-dependent methyltransferase proteins contain four conserved motifs which define the SAM-binding site (Kagan & Clarke (1994) *Arch Biochem Biophys.* 310:417-427). The four domains are referred to as motif I, post I, motif II, and motif III. The four domains are present in all of the PEAMT1-like proteins shown in FIG. 7 and all of the PEAMT2-like proteins shown in FIG. 8. Their predicted amino acid positions in the PEAMT1-like and PEAMT2-like proteins are listed in Tables 3 and 4 respectively.

Table 3: Amino Acid positions of conserved SAM-binding motifs in Nematode PEAMT1-like proteins

Nematode	Motif I	Post I	Motif II	Motif III
A. suum	56-63	76-80	114-120	143-152
H. contortus	56-63	76-80	114-120	143-152
M. incognita	64-71	84-88	122-128	151-160
S. stercoralis	56-63	76-80	118-124	147-156
C. elegans $_a$	70-77	90-94	128-134	157-166
C. elegans $_b$	79-86	99-103	137-143	166-175

Table 4: Amino Acid positions of conserved SAM-binding motifs in Nematode PEAMT2-like proteins

Nematode	Motif I	Post I	Motif II	Motif III
A. suum	230-238	252-256	292-298	319-328
M. javanica	254-262	276-280	316-322	343-352
C. elegans	228-236	250-254	290-296	317-326

The similarity between *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* PEAMT-like sequences and other sequences were also investigated by comparison to sequence databases using BLASTP analysis against nr (a non-redundant protein sequence database available at www.ncbi.nlm.nih.gov) and TBLASTN analysis against dbest (an EST sequence database available at www.ncbi.nlm.nih.gov; top 500 hits; E = 1e-4). The "Expect (E) value" is the number of sequences that are predicted to align by chance to the query sequence with a score S or greater given the size of the database queried. This analysis was used to determine the potential number of plant and vertebrate homologs for each of the nematode PEAMT-like polypeptides described above. *A. suum* (SEQ ID NO: 1 and 5), *H. contortus* (SEQ ID NO:2), *M. incognita* (SEQ ID NO:3), *M. javanica* (SEQ ID NO: 6), *S. stercoralis* (SEQ ID NO:4) and *C. elegans* (SEQ ID NO:19, 20 and 21) PEAMT-like sequences had no high scoring vertebrate hits in **nr** or **dbest** having sufficient sequence

similarity to meet the threshold E value of 1e-4 (this E value approximately corresponds to a threshold for removing sequences having a sequence identity of less than about 25% over approximately 100 amino acids). Accordingly, the *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* PEAMT-like enzymes of this invention do not appear to share significant sequence similarity with common vertebrate methyltransferase enzymes such as the *Homo sapiens* (gi|13345056|gb|AAK19172.1|[13345056]) or the *Rattus norvegicus* (gi|310195|gb|AAA03154.1|[310195]) phosphatidylethanolamine n-methyltransferase.

On the basis of the lack of similarity to vertebrate methyltransferases and the redundancy of choline biosynthesis in plants, the *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* PEAMT-like enzymes are useful targets of inhibitory compounds selective for some nematodes over their hosts (e.g., humans, animals, and plants).

Functional predictions were made using BLAST with the default parameters on the nr database. BLAST searches and multiple alignment construction with CLUSTALX demonstrated that the *C elegans* genes ZK622.3a and F54D11.1 share strong homology with the plant PEAMT genes and are therefore related to the plant PEAMT family. Reciprocal blast searches and phylogenetic trees confirm that the nucleotide sequences from *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* are orthologs of the *C. elegans* gene and are therefore likely PEAMT proteins. Protein localizations were predicted using TargetP and transmembrane domains with TMHMM. The *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* PEAMT polypeptides (SEQ ID NO: 7, 8, 9, 10, 11 and 12, respectively) are likely localized in the cytoplasm as in the case of the wheat PEAMT homolog, as they lack secretion or strong mitochondrial localization predictions and have no predicted transmembrane regions.

RNA Mediated Interference (RNAi)

A double stranded RNA (dsRNA) molecule can be used to inactivate a phosphoethanolamine *N*-methyl transferase (PEAMT) gene in a cell by a process known as RNA mediated-interference (Fire et al. (1998) *Nature* 391:806-811, and Gönczy et al. (2000) *Nature* 408:331-336). The dsRNA molecule can have the nucleotide sequence of a PEAMT nucleic acid (preferably exonic) or a fragment thereof. For example, the molecule can comprise at least 50, at least 100, at least 200, at least 300, or at least 500 or more contiguous

nucleotides of a PEAMT-like gene. The dsRNA molecule can be delivered to nematodes via direct injection, by soaking nematodes in aqueous solution containing concentrated dsRNA, or by raising bacteriovorous nematodes on *E. coli* genetically engineered to produce the dsRNA molecule (Kamath et al. (2000) *Genome Biol.* 2; Tabara et al. (1998) *Science* 282:430-431).

PEAMT RNAi by feeding:

C. elegans can be grown on lawns of *E. coli* genetically engineered to produce double-stranded RNA (dsRNA) designed to inhibit PEAMT1 or PEAMT2 expression. Briefly, *E. coli* were transformed with genomic fragments encoding portions of the *C. elegans* PEAMT1 or the PEAMT2 gene. Specifically, a 960 nucleotide fragment was amplified from the PEAMT1 gene using oligo-nucleotide primers containing the sequences 5'-ATGGTGAACGTTCGTCGTGC-3' and 5'-CATACGTATTTCTCATCATC-3' respectively, or an 854 nucleotide fragment was amplified from the PEAMT2 gene using oligo-nucleotide primers containing the sequences 5'-CCAGATTATTACCAACGCCG-3' and 5'-TGAACTTACATAGATTCTTG-3' respectively. The PEAMT1 and PEAMT2 genomic fragments were cloned separately into an *E. coli* expression vector between opposing T7 polymerase promoters. The clone was then transformed into a strain of *E. coli* that carries an IPTG-inducible T7 polymerase. As a control, *E. coli* was transformed with a gene encoding the Green Fluorescent Protein (GFP). Feeding RNAi was initiated from *C. elegans* larvae at 23 °C on NGM plates containing IPTG and *E. coli* expressing the *C. elegans* PEAMT1 or PEAMT2, or GFP dsRNA. If the starting worm (the P0) was an L1, or a dauer larva, the phenotype of both the PEAMT1 and PEAMT2 RNAi-generated mutants was complete or almost complete sterility. One the other hand, if the P0 animal was an L4 larva, then the phenotype of both the PEAMT1 and PEAMT2 RNAi-generated mutants was L1/L2 larval arrested development and lethality. The sequence of the PEAMT1 and PEAMT2 genes is of sufficiently high complexity (i.e., unique) such that the RNAi is not likely to represent cross reactivity with other genes.

C. elegans cultures grown in the presence of *E. coli* expressing dsRNA from the PEAMT1 or the PEAMT2 gene were strongly impaired indicating that the PEAMT genes provide essential functions in nematodes and that dsRNA from the PEAMT-like genes is

lethal when ingested by *C. elegans*. These results demonstrate that PEAMT's are important for the viability of *C. elegans* and suggest that they are useful targets for the development of compounds that reduce the viability of nematodes.

Chemical rescue of the PEAMT1 and PEAMT2 RNAi-generated phenotype.

The experiments described below were designed to test whether the PEAMT1/PEAMT2 RNAi knockout phenotype can be rescued by providing *C. elegans* with the products downstream of the predicted PEAMT reaction catalyzed by the enzymes. The free bases (EA, MME, DME and Cho) were added to the bacterial medium and it was assumed that these would be taken up and converted to the corresponding phosphobases by the actions of ethanolamine/choline kinases.

C. elegans worms were fed bacteria expressing dsRNA homologous to PEAMT1, PEAMT2, actin, or GFP along with specific chemicals (EA, MME, DME or Cho). Chemicals were added to NGM plates at various concentrations and negative (GFP dsRNA) and positive (actin dsRNA) controls were performed for each chemical or chemical mixture at each concentration. Specifically, agar plates containing NGM and the chemicals specified in Table 1 (see below) were seeded with bacteria expressing double-stranded RNA homologous to either PEAMT1 or PEAMT2. In some experiments a single L1 or dauer larva was placed on each plate, and the P0 and the F1 were examined for the next 5 days. In other experiments, a single L4 *C. elegans* hermaphrodite was placed on each plate. The hermaphrodite was allowed to lay eggs for 24 hours and the phenotype of the F1 progeny was scored 48 hours after the initial 24-hour egg-laying period. At the time of scoring, 4 individual F1 progeny were cloned to separate plates containing the same chemical and bacteria they were grown on. The F1 and F2 progeny were examined over the next 4-5 days for the presence of a phenotype.

Table 5: *C. elegans* PEAMT1 and PEAMT2 RNAi feeding phenotypes (starting with *C. elegans* L1, dauer, or L4 larva as the P0 animal).

P0	Compounds added to the plate media	F1 phenotype	
		PEAMT1 dsRNA	PEAMT2 dsRNA

L1	None	Sterility	Sterility
	10 mM DME	**Fertile adults**	Sterility
Dauer	None	Partial sterility	Partial sterility
	10 mM DME	**Fertile adults**	Sterility
L4	None	L1/L2 arrest/lethality	L1/L2 arrest/lethality
	10 mM ethanolamine (EA)	L1/L2 arrest/lethality	L1/L2 arrest/lethality
	5 or 10 mM MME	**Fertile adults**	L1/L2 arrest/lethality
	5 or 10 mM DME	**Fertile adults**	L1/L2 arrest/lethality
	5 mM choline (Cho)	L1/L2 arrest/lethality	L1/L2 arrest/lethality
	10 or 15 mM Cho	Sterile adults	L1/L2 arrest/lethality
	25 mM or 30 mM Cho	**Fertile adults**	**Fertile adults**
	5 mM each EA, MME 5 mM each EA, DME 5 mM each EA, Cho 5 mM each MME, DME 5 mM each MME, Cho 5 mM each DME, Cho 5 mM each MME, DME, Cho	**Fertile adults**	L1/L2 arrest/lethality

The *C. elegans* phosphoethanolamine *N*-methyltransferase proteins PEAMT1 and PEAMT2 together catalyze the conversion of phosphoethanolamine to phosphocholine. The RNAi-generated mutants of PEAMT1 or PEAMT2 are both predicted to have decreased levels of choline which leads to sterility, or L1/L2 larval arrested development and death. Addition of 25 mM choline rescues the larval arrest associated with both PEAMT1 and PEAMT2 RNAi phenotypes. However, only the PEAMT1 mutants are rescued by the addition of 5 mM monomethylethanolamine (MME) or 5 mM dimethylethanolamine (DME) while the PEAMT2 mutants are not (see Table 5). These data are consistent with the prediction that PEAMT1 catalyzes the first methylation while PEAMT2 catalyzes the second and third methylations in the conversion of pEA to pCho:

pEA → **pMME** → **pDME** → **pCho**

PEAMT1 PEAMT2 PEAMT2

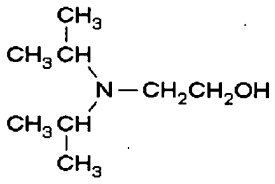
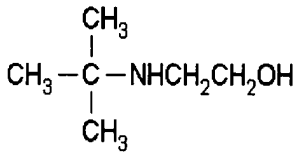
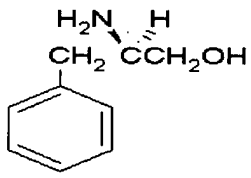
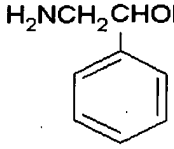
Five mM DME rescues the sterility associated with PEAMT1 RNAi. The rescue by DME strongly suggests the sterility is due to a reduction in choline production and not due to other changes caused by the PEAMT mutations.

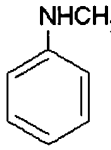
The data also demonstrate that when choline alone is used as the rescuing chemical, 25 mM choline is required to complement the PEAMT1 and PEAMT2 RNAi phenotypes. This suggests that chemicals that interfere with this pathway will not likely be counteracted by the amount of choline nematodes can acquire from the environment.

Nematicidal Activity of Small Molecules Structurally Similar to Ethanolamine Against *Caenorhabditis elegans*

The structures of ethanolamine-like molecules tested against *C. elegans* for nematicidal activity are shown below.

Table 6:

COMPOUND	STRUCTURE
2-(diisopropylamino)ethanol (N-substituted)	
2-(tert-butylamino)ethanol (N-substituted)	
D-phenylalaninol (C2-subsitituted)	
2-amino-1-phenylethanol (C1-subsitituted)	

N-(2-hydroxyethyl)aniline (N-substituted)	

One approach to the development of chemicals that interfere with the function of an enzyme is to identify compounds that mimic substrate binding but that cannot be acted on by the enzyme. Therefore, several ethanolamine-derived compounds were tested for the ability to kill *C. elegans* in culture. Compounds with substitutions at various positions on ethanolamine were tested including some with substitutions on the nitrogen, the carbon adjacent to the nitrogen (C2), and on the carbon adjacent to the oxygen (C1).

A single *C. elegans* L4 larva (the P0 animal) was placed on a lawn of *E. coli* that had been spotted onto NGM plates containing various concentrations of the ethanolamine-like compounds. The growth and development of the P0 and its F1 progeny at 23 °C was monitored by visual observation over several days. Four of the compounds tested [2-(diisopropylamino)ethanol, 2-(tert-butylamino)ethanol, D-phenylalaninol and N-(2-hydroxyethyl)aniline], showed nematicidal activity against *C. elegans*. In addition, the phenotype of worms treated with the nematicidal ethanolamine-like compounds mimicked the RNAi-phenotype of PEAMT1 and PEAMT2. That is, the F1 progeny of the treated worm did not develop beyond the L1/L2 stage and died. Treatment of *C. elegans* with the C1-substituted compound 2-amino-1-phenylethanol showed no nematicidal effect.

Table 7: Nematicidal activity of ethanolamine-like compounds against *C. elegans*.

COMPOUND	CONCENTRATION	F1 PHENOTYPE
2-(diisopropylamino)ethanol	10 mM	L1/L2 arrest/lethality
2-(tert-butylamino)ethanol	10 mM	L1/L2 arrest/lethality
D-phenylalaninol	10 mM	L1/L2 arrest/lethality
2-amino-1-phenylethanol	25 mM	Wild-type development
N-(2-hydroxyethyl)aniline	10 mM	L1/L2 arrest/lethality
Control (no compound)	Not applicable	Wild-type development

Nematicidal activity of ethanolamine-like compounds against plant parasites.

The ethanolamine-like compounds mentioned above are also nematicidal against the plant parasites *Meloidogyne incongnita* and *Meloidogyne javanica*. Between 25 and 50 J2 stage larvae were soaked in the compounds for 48 hours at the indicated concentrations. After the treatment, the larvae were moved to an agar plate containing NGM. Worms that crawl away from the application spot are scored as alive while those that remain at the application spot are scored as dead. The three compounds that were nematicidal against *C. elegans* were also nematicidal against *M. incognita* and *M. javanica*

Table 8:

COMPOUND	SPECIES	CONCENTRATION	% DEAD WORMS
di-isopropylethanolamine	*M. incognita*	2.5 mM	75
	M. javanica	2.5 mM	70
2-(tert-butylamino)ethanol	*M. incognita*	5 mM	50
	M. javanica	15 mM	50
D-phenylalaninol	*M. incognita*	25 mM	100
Control (no compound)	*M. incognita*	not applicable	15

Nematicidal activity of ethanolamine-like compounds against other nematodes.

The ethanolamine-like compounds mentioned above are also nematicidal against *Acrobiloides ellesmerensis* and *Cephalobus sp.* Assays were done as those described for *C. elegans* L4 larvae. Three of the four compounds that were nematicidal against *C. elegans* were tested and were found to be nematicidal against *A. ellesmerensis* and *Cephalobus sp.*

Table 9:

COMPOUND	SPECIES	CONCENTRATION	F1 PHENOTYPE
diisopropylamino)ethanol	*A. ellesmerensis*	10 mM	L1/L2 arrest/lethality
	Cephalobus sp.	10 mM	L1/L2 arrest/lethality
2-(tert-	*A. ellesmerensis*	10 mM	L1/L2 arrest/lethality

butylamino)ethanol	*Cephalobus sp.*	10 mM	L1/L2 arrest/lethality
D-phenylalaninol	*A. ellesmerensis*	12.5 mM	L1/L2 arrest/lethality
	Cephalobus sp.	12.5 mM	L1/L2 arrest/lethality
Control (no compound)	*Cephalobus sp.*	not applicable	Wild-type

Sulfonic, phosphonic, or phosphate prodrugs based on the structures of the molecules discussed here will provide better activity than the parent molecules themselves. Enzymes like PEAMT1 and PEAMT2, which interact with phosphorylated substrates, bind more tightly to the phosphorylated forms of the substrate than to the non-phosphorylated forms. For example, in the case of SH2 domains, phosphorylated peptides exhibit binding four orders of magnitude greater than non-phosphorylated peptides (Bradshaw et al, (1999) *J. Mol. Biol.* 293(4):971-85). Therefore, the addition of a phosphate, or a phosphate mimic (e.g., phosphonate, sulfonate) to the ethanolamine-like compounds will increase the affinity for the enzyme making them more potent inhibitors of the PEAMT enzymes.

Identification of Additional Phosphoethanolamine n-Methyltransferase-Like Sequences

A skilled artisan can utilize the methods provided in the example above to identify additional nematode phosphoethanolamine n-methyltransferase-like sequences, e.g., PEAMT-like sequence from nematodes other than *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* and/or *C. elegans*. In addition, nematode PEAMT-like sequences can be identified by a variety of methods including computer-based database searches, hybridization-based methods, and functional complementation.

Database Identification. A nematode phosphoethanolamine n-methyltransferase-like sequence can be identified from a sequence database, e.g., a protein or nucleic acid database using a sequence disclosed herein as a query. Sequence comparison programs can be used to compare and analyze the nucleotide or amino acid sequences. One such software package is the BLAST suite of programs from the National Center for Biotechnology Institute (NCBI; Altschul et al. (1997) *Nucl. Acids Research* 25:3389-3402). A phosphoethanolamine n-methyltransferase -like sequence of the invention can be used to query a sequence database, such as nr, dbest (expressed sequence tag (EST) sequences), and htgs (high-throughput genome sequences), using a computer-based search, e.g., FASTA,

BLAST, or PSI-BLAST search. Homologous sequences in other species (e.g., plants and animals) can be detected in a PSI-BLAST search of a database such as nr (E value = 10, H value = 1e-2, using, for example, four iterations; available at www.ncbi.nlm.nih.gov). Sequences so obtained can be used to construct a multiple alignment, e.g., a ClustalX alignment, and/or to build a phylogenetic tree, e.g., in ClustalX using the Neighbor-Joining method (Saitou et al. (1987) *Mol. Biol. Evol.* 4:406-425) and bootstrapping (1000 replicates; Felsenstein (1985) *Evolution* 39:783-791). Distances may be corrected for the occurrence of multiple substitutions [$D_{corr} = -\ln(1-D-D^2/5)$ where D is the fraction of amino acid differences between two sequences] (Kimura (1983) *The Neutral Theory of Molecular Evolution*, Cambridge University Press).

The aforementioned search strategy can be used to identify phosphoethanolamine n-methyltransferase-like sequences in nematodes of the following non-limiting, exemplary genera: Plant-parasitic nematode genera: *Afrina, Anguina, Aphelenchoides, Belonolaimus, Bursaphelenchus, Cacopaurus, Cactodera, Criconema, Criconemoides, Cryphodera, Ditylenchus, Dolichodorus, Dorylaimus, Globodera, Helicotylenchus, Hemicriconemoides, Hemicycliophora, Heterodera, Hirschmanniella, Hoplolaimus, Hypsoperine, Longidorus, Meloidogyne, Mesoanguina, Nacobbus, Nacobbodera, Panagrellus, Paratrichodorus, Paratylenchus, Pratylenchus, Pterotylenchus, Punctodera, Radopholus, Rhadinaphelenchus, Rotylenchulus, Rotylenchus, Scutellonema, Subanguina, Thecavermiculatus, Trichodorus, Turbatrix, Tylenchorhynchus, Tylenchulus, Xiphinema.*

Animal- and human-parasitic nematode genera: *Acanthocheilonema, Aelurostrongylus, Ancylostoma, Angiostrongylus, Anisakis, Ascaris, Ascarops, Bunostomum, Brugia, Capillaria, Chabertia, Cooperia, Crenosoma, Cyathostome species (Small Strongyles), Dictyocaulus, Dioctophyma, Dipetalonema, Dirofiliaria, Dracunculus, Draschia, Elaneophora, Enterobius, Filaroides, Gnathostoma, Gonylonema, Habronema, Haemonchus, Hyostrongylus, Lagochilascaris, Litomosoides, Loa, Mammomonogamus, Mansonella, Muellerius, Metastrongylid, Necator, Nematodirus, Nippostrongylus, Oesophagostomum, Ollulanus, Onchocerca, Ostertagia, Oxyspirura, Oxyuris, Parafilaria, Parascaris, Parastrongyloides, Parelaphostrongylus, Physaloptera, Physocephalus, Protostrongylus, Pseudoterranova, Setaria, Spirocerca, Stephanurus, Stephanofilaria,*

Strongyloides, Strongylus, Spirocerca, Syngamus, Teladorsagia, Thelazia, Toxascaris, Toxocara, Trichinella, Trichostrongylus, Trichuris, Uncinaria, and *Wuchereria.*

Particularly preferred nematode genera include: Plant: *Anguina, Aphelenchoides, Belonolaimus, Bursaphelenchus, Ditylenchus, Dolichodorus, Globodera, Heterodera, Hoplolaimus, Longidorus, Meloidogyne, Nacobbus, Pratylenchus, Radopholus, Rotylenchus, Tylenchulus, Xiphinema.*

Animal and human parasites: *Ancylostoma, Ascaris, Brugia, Capillaria, Cooperia, Cyathostome species, Dictyocaulus, Dirofiliaria, Dracunculus, Enterobius, Haemonchus, Necator, Nematodirus, Oesophagostomum, Onchocerca, Ostertagia, Oxyspirura, Oxyuris, Parascaris, Strongyloides, Strongylus, Syngamus, Teladorsagia, Thelazia, Toxocara, Trichinella, Trichostrongylus, Trichuris,* and *Wuchereria.*

Particularly preferred nematode species include: Plant parasites: *Anguina tritici, Aphelenchoides fragariae, Belonolaimus longicaudatus, Bursaphelenchus xylophilus, Ditylenchus destructor, Ditylenchus dipsaci Dolichodorus heterocephalous, Globodera pallida, Globodera rostochiensis, Globodera tabacum, Heterodera avenae, Heterodera cardiolata, Heterodera carotae, Heterodera cruciferae, Heterodera glycines, Heterodera major, Heterodera schachtii, Heterodera zeae, Hoplolaimus tylenchiformis, Longidorus sylphus, Meloidogyne acrnea, Meloidogyne arenaria, Meloidogyne chitwoodi, Meloidogyne exigua, Meloidogyne graminicola, Meloidogyne hapla, Meloidogyne incognita, Meloidogyne javanica, Meloidogyne nassi, Nacobbus batatiformis, Pratylenchus brachyurus, Pratylenchus coffeae, Pratylenchus penetrans, Pratylenchus scribneri, Pratylenchus zeae, Radopholus similis, Rotylenchus reniformis, Tylenchulus semipenetrans, Xiphinema americanum.*

Animal and human parasites: *Ancylostoma braziliense, Ancylostoma caninum , Ancylostoma ceylanicum , Ancylostoma duodenale, Ancylostoma tubaeforme, Ascaris suum, Ascaris lumbrichoides, Brugia malayi, Capillaria bovis, Capillaria plica, Capillaria feliscati, Cooperia oncophora, Cooperia punctata, Cyathostome species, Dictyocaulus filaria, Dictyocaulus viviparus, Dictyocaulus arnfieldi, Dirofiliaria immitis, Dracunculus insignis, Enterobius vermicularis, Haemonchus contortus, Haemonchus placei, Necator americanus, Nematodirus helvetianus, Oesophagostomum radiatum, Onchocerca volvulus, Onchocerca cervicalis, Ostertagia ostertagi, Ostertagia circumcincta, Oxyuris equi, Parascaris equorum, Strongyloides stercoralis, Strongylus vulgaris, Strongylus edentatus, Syngamus trachea,*

Teladorsagia circumcincta, Toxocara cati, Trichinella spiralis, Trichostrongylus axei, Trichostrongylus colubriformis, Trichuris vulpis, Trichuris suis, Trichurs trichiura, and *Wuchereria bancrofti.*

Further, a phosphoethanolamine n-methyltransferase-like sequence can be used to identify additional PEAMT-like sequence homologs within a genome. Multiple homologous copies of a PEAMT-like sequence can be present. For example, a nematode PEAMT-like sequence can be used as a seed sequence in an iterative PSI-BLAST search (default parameters, substitution matrix = Blosum62, gap open = 11, gap extend = 1) of a non redundant database such as **wormpep** (E value=1e-2, H value = 1e-4, using, for example 4 iterations) to determine the number of homologs in a database, e.g., in a database containing the complete genome of an organism. A nematode PEAMT-like sequence can be present in a genome along with 1, 2, 3, 4, 5, 6, 8, 10, or more homologs.

Hybridization Methods. A nematode phosphoethanolamine n-methyltransferase-like sequence can be identified by a hybridization-based method using a sequence provided herein as a probe. For example, a library of nematode genomic or cDNA clones can be hybridized under low stringency conditions with the probe nucleic acid. Stringency conditions can be modulated to reduce background signal and increase signal from potential positives. Clones so identified can be sequenced to verify that they encode PEAMT-like sequences.

Another hybridization-based method utilizes an amplification reaction (e.g., the polymerase chain reaction (PCR)). Oligonucleotides, e.g., degenerate oligonucleotides, are designed to hybridize to a conserved region of a PEAMT-like sequence (e.g., a region conserved in the three nematode sequences depicted in FIG. 3). The oligonucleotides are used as primers to amplify a PEAMT-like sequence from template nucleic acid from a nematode, e.g., a nematode other than *A. suum, H. contortus, M. incognita, M. javanica* and *S. stercoralis* and/or *C. elegans*. The amplified fragment can be cloned and/or sequenced.

Complementation Methods. A nematode phosphoethanolamine n-methyltransferase -like sequence can be identified from a complementation screen for a nucleic acid molecule that restores PEAMT-like activity to a cell lacking a PEAMT-like activity. Routine methods can be used to construct strains (i.e., nematode strains) that lack specific enzymatic activities, e.g., PEAMT activity. For example, a nematode strain mutated at the PEAMT gene locus

can be identified by selecting for resistance to inhibitory compounds. Such a strain can be transformed with a plasmid library expressing nematode cDNAs. Strains can be identified in which PEAMT activity is restored. For example, the PEAMT mutant strains transformed with the plasmid library can be exposed to allosteric inhibitors or other inhibitory compounds to select for strains that have acquired sensitivity to the inhibitors and are expressing a nematode PEAMT-like gene. The plasmid harbored by the strain can be recovered to identify and/or characterize the inserted nematode cDNA that provides PEAMT-like activity when expressed.

Full-length cDNA and Sequencing Methods. The following methods can be used, e.g., alone or in combination with another method described herein, to obtain full-length nematode PEAMT-like genes and determine their sequences.

Plant parasitic nematodes are maintained on greenhouse pot cultures depending on nematode preference. Root Knot Nematodes (*Meloidogyne* sp) are propagated on Rutgers tomato (Burpee), while Soybean Cyst Nematodes (*Heterodera* sp) are propagated on soybean. Total nematode RNA is isolated using the TRIZOL reagent (Gibco BRL). Briefly, 2 ml of packed worms are combined with 8 ml TRIZOL reagent and solubilized by vortexing. Following 5 minutes of incubation at room temperature, the samples are divided into smaller volumes and spun at 14,000 x g for 10 minutes at 4 °C to remove insoluble material. The liquid phase is extracted with 200 µl of chloroform, and the upper aqueous phase is removed to a fresh tube. The RNA is precipitated by the addition of 500 µl of isopropanol and centrifuged to pellet. The aqueous phase is carefully removed, and the pellet is washed in 75% ethanol and spun to re-collect the RNA pellet. The supernatant is carefully removed, and the pellet is air dried for 10 minutes. The RNA pellet is resuspended in 50 µl of DEPC-H_2O and analyzed by spectrophotometry at λ 260 and 280 nm to determine yield and purity. Yields can be 1-4 mg of total RNA from 2 ml of packed worms.

Full-length cDNAs can be generated using 5' and 3' RACE techniques in combination with EST sequence information. The molecular technique 5' RACE (Life Technologies, Inc., Rockville, MD) can be employed to obtain complete or near-complete 5' ends of cDNA sequences for nematode PEAMT-like cDNA sequences. Briefly, following the instructions provided by Life Technologies, first strand cDNA is synthesized from total nematode RNA using Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) and a

gene specific "antisense" primer, e.g., designed from available EST sequence. RNase H is used to degrade the original mRNA template. The first strand cDNA is separated from unincorporated dNTPs, primers, and proteins using a GlassMAX Spin Cartridge. Terminal deoxynucleotidyl transferase (TdT) is used to generate a homopolymeric dC tailed extension by the sequential addition of dCTP nucleotides to the 3' end of the first strand cDNA. Following addition of the dC homopolymeric extension, the first strand cDNA is directly amplified without further purification using Taq DNA polymerase, a gene specific "antisense" primer designed from available EST sequences to anneal to a site located within the first strand cDNA molecule, and a deoxyinosine-containing primer that anneals to the homopolymeric dC tailed region of the cDNA in a polymerase chain reaction (PCR). 5' RACE PCR amplification products are cloned into a suitable vector for further analysis and sequencing.

The molecular technique, 3' RACE (Life Technologies, Inc., Rockville, MD), can be employed to obtain complete or near-complete 3' ends of cDNA sequences for nematode PEAMT-like cDNA sequences. Briefly, following the instructions provided by Life Technologies (Rockville, MD), first strand cDNA synthesis is performed on total nematode RNA using SuperScript™ Reverse Transcriptase and an oligo-dT primer that anneals to the polyA tail. Following degradation of the original mRNA template with RNase H, the first strand cDNA is directly PCR amplified without further purification using Taq DNA polymerase, a gene specific primer designed from available EST sequences to anneal to a site located within the first strand cDNA molecule, and a "universal" primer which contains sequence identity to 5' end of the oligo-dT primer. 3' RACE PCR amplification products are cloned into a suitable vector for further analysis and sequencing.

Nucleic Acid Variants

Isolated nucleic acid molecules of the present invention include nucleic acid molecules that have an open reading frame encoding a PEAMT-like polypeptide. Such nucleic acid molecules include molecules having: the sequences recited in SEQ ID NO: 1, 2, 3, 4, 5 and/or 6; and sequences coding for the PEAMT-like proteins recited in SEQ ID NO: 13, 14, 15, 16, 17 and/or 18. These nucleic acid molecules can be used, for example, in a hybridization assay to detect the presence of a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* nucleic acid in a sample.

The present invention includes nucleic acid molecules such as those shown in SEQ ID NO: 1, 2, 3, 4, 5 and/or 6 that may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions, or insertions. Nucleotide insertional derivatives of the nematode gene of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence, although random insertion is also possible with suitable screening of the resulting product. Deletion variants are characterized by the removal of one or more nucleotides from the sequence. Nucleotide substitution variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be silent (e.g., synonymous), meaning that the substitution does not alter the amino acid defined by the codon. Alternatively, substitutions are designed to alter one amino acid for another amino acid (e.g., non-synonymous). A non-synonymous substitution can be conservative or non-conservative. A substitution can be such that activity, e.g., a PEAMT-like activity, is not impaired. A conservative amino acid substitution results in the alteration of an amino acid for a similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity, e.g., an amino acid substitution listed in Table 10 below. At some positions, even conservative amino acid substitutions can disrupt the activity of the polypeptide.

Table 10: Conservative Amino Acid Replacements

Amino acid	Code	Replace with any of
Alanine	Ala	Gly, Cys, Ser
Arginine	Arg	Lys, His
Asparagine	Asn	Asp, Glu, Gln,
Aspartic Acid	Asp	Asn, Glu, Gln
Cysteine	Cys	Met, Thr, Ser
Glutamine	Gln	Asn, Glu, Asp
Glutamic Acid	Glu	Asp, Asn, Gln
Glycine	Gly	Ala
Histidine	His	Lys, Arg
Isoleucine	Ile	Val, Leu, Met
Leucine	Leu	Val, Ile, Met
Lysine	Lys	Arg, His
Methionine	Met	Ile, Leu, Val
Phenylalanine	Phe	Tyr, His, Trp
Proline	Pro	
Serine	Ser	Thr, Cys, Ala
Threonine	Thr	Ser, Met, Val
Tryptophan	Trp	Phe, Tyr
Tyrosine	Tyr	Phe, His
Valine	Val	Leu, Ile, Met

The current invention also embodies splice variants of nematode PEAMT-like sequences.

Another aspect of the present invention embodies a polypeptide-encoding nucleic acid molecule that is capable of hybridizing under conditions of low stringency (or high stringency) to the nucleic acid molecule put forth in SEQ ID NO: 1, 2, 3, 4, 5 and/or 6, or their complements.

The nucleic acid molecules that encode for phosphoethanolamine n-methyltransferase-like polypeptides may correspond to the naturally occurring nucleic acid molecules or may differ by one or more nucleotide substitutions, deletions, and/or additions. Thus, the present invention extends to genes and any functional mutants, derivatives, parts, fragments, naturally occurring polymorphisms, homologs or analogs thereof or non-functional molecules. Such nucleic acid molecules can be used to detect polymorphisms of PEAMT genes or PEAMT-like genes, e.g., in other nematodes. As mentioned below, such molecules are useful as genetic probes; primer sequences in the enzymatic or chemical synthesis of the gene; or in the generation of immunologically interactive recombinant

molecules. Using the information provided herein, such as the nucleotide sequence SEQ ID NO: 1, 2, 3, 4, 5 and/or 6, a nucleic acid molecule encoding a PEAMT-like molecule may be obtained using standard cloning and a screening techniques, such as a method described herein.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, or in the form of DNA, including, for example, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. The nucleic acids may be in the form of RNA/DNA hybrids. Single-stranded DNA or RNA can be the coding strand, also referred to as the sense strand, or the non-coding strand, also known as the anti-sense strand.

One embodiment of the present invention includes a recombinant nucleic acid molecule, which includes at least one isolated nucleic acid molecule depicted in SEQ ID NO: 1, 2, 3, 4, 5, and/or 6, inserted in a vector capable of delivering and maintaining the nucleic acid molecule into a cell. The DNA molecule may be inserted into an autonomously replicating vector (suitable vectors include, for example, pGEM3Z and pcDNA3, and derivatives thereof). The vector nucleic acid may be a bacteriophage DNA such as bacteriophage lambda or M13 and derivatives thereof. The vector may be either RNA or DNA, single- or double-stranded, prokaryotic, eukaryotic, or viral. Vectors can include transposons, viral vectors, episomes, (e.g., plasmids), chromosomes inserts, and artificial chromosomes (e.g. BACs or YACs). Construction of a vector containing a nucleic acid described herein can be followed by transformation of a host cell such as a bacterium. Suitable bacterial hosts include, but are not limited to, *E. coli*. Suitable eukaryotic hosts include yeast such as *S. cerevisiae*, other fungi, vertebrate cells, invertebrate cells (e.g., insect cells), plant cells, human cells, human tissue cells, and whole eukaryotic organisms. (e.g., a transgenic plant or a transgenic animal). Further, the vector nucleic acid can be used to generate a virus such as vaccinia or baculovirus.

The present invention also extends to genetic constructs designed for polypeptide expression. Generally, the genetic construct also includes, in addition to the encoding nucleic acid molecule, elements that allow expression, such as a promoter and regulatory sequences. The expression vectors may contain transcriptional control sequences that control transcriptional initiation, such as promoter, enhancer, operator, and repressor sequences. A

variety of transcriptional control sequences are well known to those in the art and may be functional in, but are not limited to, a bacterium, yeast, plant, or animal cell. The expression vector can also include a translation regulatory sequence (e.g., an untranslated 5' sequence, an untranslated 3' sequence, a poly A addition site, or an internal ribosome entry site), a splicing sequence or splicing regulatory sequence, and a transcription termination sequence. The vector can be capable of autonomous replication or it can integrate into host DNA.

In an alternative embodiment, the DNA molecule is fused to a reporter gene such as β-glucuronidase gene, β-galactosidase (lacZ), chloramphenicol-acetyltransferase gene, a gene encoding green fluorescent protein (and variants thereof), or red fluorescent protein firefly luciferase gene, among others. The DNA molecule can also be fused to a nucleic acid encoding a polypeptide affinity tag, e.g. glutathione S-transferase (GST), maltose E binding protein, protein A, FLAG tag, hexa-histidine, or the influenza HA tag. The affinity tag or reporter fusion joins the reading frames of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6 to the reading frame of the reporter gene encoding the affinity tag such that a translational fusion is generated. Expression of the fusion gene results in translation of a single polypeptide that includes both a nematode PEAMT-like region and reporter protein or affinity tag. The fusion can also join a fragment of the reading frame of SEQ ID NO: 1, 2, 3, 4, 5 and/or 6. The fragment can encode a functional region of the PEAMT-like polypeptides, a structurally intact domain, or an epitope (e.g., a peptide of about 8, 10, 20, or 30 or more amino acids). A nematode PEAMT-like nucleic acid that includes at least one of a regulatory region (e.g., a 5' regulatory region, a promoter, an enhancer, a 5' untranslated region, a translational start site, a 3' untranslated region, a polyadenylation site, or a 3' regulatory region) can also be fused to a heterologous nucleic acid. For example, the promoter of a PEAMT-like nucleic acid can be fused to a heterologous nucleic acid, e.g., a nucleic acid encoding a reporter protein.

Suitable cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. A transformed cell of the present invention is also herein referred to as a recombinant or transgenic cell. Suitable cells can either be untransformed cells or cells that have already been transformed with at least one nucleic acid molecule. Suitable cells for transformation according to the present invention can either be: (i) endogenously capable of expressing the PEAMT-like protein or; (ii) capable of producing

such protein after transformation with at least one nucleic acid molecule of the present invention.

In an exemplary embodiment, a nucleic acid of the invention is used to generate a transgenic nematode strain, e.g., a transgenic *C. elegans* strain. To generate such a strain, nucleic acid is injected into the gonad of a nematode, thus generating a heritable extrachromosomal array containing the nucleic acid (see, e.g., Mello et al. (1991) *EMBO J.* 10:3959-3970). The transgenic nematode can be propagated to generate a strain harboring the transgene. Nematodes of the strain can be used in screens to identify inhibitors specific for a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* PEAMT-like gene.

Oligonucleotides

Also provided are oligonucleotides that can form stable hybrids with a nucleic acid molecule of the present invention. The oligonucleotides can be about 10 to 200 nucleotides, about 15 to 120 nucleotides, or about 17 to 80 nucleotides in length, e.g., about 10, 20, 30, 40, 50, 60, 80, 100, 120 nucleotides in length. The oligonucleotides can be used as probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit nematode PEAMT-like protein activity or production (e.g., antisense, triplex formation, ribozyme, and/or RNA drug-based reagents). The present invention includes oligonucleotides of RNA (ssRNA and dsRNA), DNA, or derivatives of either. The invention extends to the use of such oligonucleotides to protect non-nematode organisms (for example e.g., plants and animals) from disease by reading the viability of infecting namatodes, e.g., using a technology described herein. Appropriate oligonucleotide-containing therapeutic compositions can be administered to a non-nematode organism using techniques known to those skilled in the art, including, but not limited to, transgenic expression in plants or animals.

Primer sequences can be used to amplify a phosphoethanolamine n-methyltransferase-like nucleic acid or fragment thereof. For example, at least 10 cycles of PCR amplification can be used to obtain such an amplified nucleic acid. Primers can be at least about 8-40, 10-30 or 14-25 nucleotides in length, and can anneal to a nucleic acid "template molecule", e.g., a template molecule encoding an PEAMT-like genetic sequence, or a functional part thereof, or its complementary sequence. The nucleic acid primer

molecule can be any nucleotide sequence of at least 10 nucleotides in length derived from, or contained within sequences depicted in SEQ ID NO: 1, 2, 3, 4, 5 and/or 6 and their complements. The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, plant cell, fungal cell, or bacterial cell. A primer can be chemically synthesized by routine methods.

This invention embodies any phosphoethanolamine n-methyltransferase-like sequences that are used to identify and isolate similar genes from other organisms, including nematodes, prokaryotic organisms, and other eukaryotic organisms, such as other animals and/or plants.

In another embodiment, the invention provides oligonucleotides that are specific for a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* PEAMT-like nucleic acid molecule. Such oligonucleotides can be used in a PCR test to determine if a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* nucleic acid is present in a sample, e.g., to monitor a disease caused *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis.*

Protein Production

Isolated phosphoethanolamine n-methyltransferase-like proteins from nematodes can be produced in a number of ways, including production and recovery of the recombinant proteins and/or chemical synthesis of the protein. In one embodiment, an isolated nematode PEAMT-like protein is produced by culturing a cell, e.g., a bacterial, fungal, plant, or animal cell, capable of expressing the protein, under conditions for effective production and recovery of the protein. The nucleic acid can be operably linked to a heterologous promoter, e.g., an inducible promoter or a constitutive promoter. Effective growth conditions are typically, but not necessarily, in liquid media comprising salts, water, carbon, nitrogen, phosphate sources, minerals, and other nutrients, but may be any solution in which PEAMT-like proteins may be produced.

In one embodiment, recovery of the protein may refer to collecting the growth solution and need not involve additional steps of purification. Proteins of the present invention, however, can be purified using standard purification techniques, such as, but not limited to, affinity chromatography, thermaprecipitation, immunoaffinity chromatography,

ammonium sulfate precipitation, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, and others.

The phosphoethanolamine n-methyltransferase-like polypeptide can be fused to an affinity tag, e.g., a purification handle (e.g., glutathione-S-reductase, hexa-histidine, maltose binding protein, dihydrofolate reductases, or chitin binding protein) or an epitope tag (e.g., c-myc epitope tag, FLAG™ tag, or influenza HA tag). Affinity tagged and epitope tagged proteins can be purified using routine art-known methods.

Antibodies Against Phosphoethanolamine n-Methyltransferase-like Polypeptides

Recombinant phosphoethanolamine n-methyltransferase-like gene products or derivatives thereof can be used to produce immunologically interactive molecules, such as antibodies, or functional derivatives thereof. Useful antibodies include those that bind to a polypeptide that has substantially the same sequence as the amino acid sequences recited in SEQ ID NO: 7, 8, 9, 10, 11 and/or 12, or that has at least 60% similarity over 50 or more amino acids to these sequences. In a preferred embodiment, the antibody specifically binds to a polypeptide having the amino acid sequence recited in SEQ ID NO: 7, 8, 9, 10, 11 and/or 12. The antibodies can be antibody fragments and genetically engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to more than one epitope. Such antibodies may be polyclonal or monoclonal and may be selected from naturally occurring antibodies or may be specifically raised to a recombinant PEAMT-like protein.

Antibodies can be derived by immunization with a recombinant or purified PEAMT-like gene or gene product. As used herein, the term "antibody" refers to an immunoglobulin, or fragment thereof. Examples of antibody fragments include F(ab) and $F(ab')_2$ fragments, particularly functional ones able to bind epitopes. Such fragments can be generated by proteolytic cleavage, e.g., with pepsin, or by genetic engineering. Antibodies can be polyclonal, monoclonal, or recombinant. In addition, antibodies can be modified to be chimeric, or humanized. Further, an antibody can be coupled to a label or a toxin.

Antibodies can be generated against a full-length PEAMT-like protein, or a fragment thereof, e.g., an antigenic peptide. Such polypeptides can be coupled to an adjuvant to improve immunogenicity. Polyclonal serum is produced by injection of the antigen into a laboratory animal such as a rabbit and subsequent collection of sera. Alternatively, the

antigen is used to immunize mice. Lymphocytic cells are obtained from the mice and fused with myelomas to form hybridomas producing antibodies.

Peptides for generating phosphoethanolamine n-methyltransferase-like antibodies can be about 8, 10, 15, 20, 30 or more amino acid residues in length, e.g., a peptide of such length obtained from SEQ ID NO: 7, 8, 9, 10, 11 and/or 12. Peptides or epitopes can also be selected from regions exposed on the surface of the protein, e.g., hydrophilic or amphipathic regions. An epitope in the vicinity of the active or binding site can be selected such that an antibody binding such an epitope would block access to the active site or prevent binding. Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided. An antibody to a PEAMT-like protein can modulate a PEAMT-like activity.

Monoclonal antibodies, which can be produced by routine methods, are obtained in abundance and in homogenous form from hybridomas formed from the fusion of immortal cell lines (e.g., myelomas) with lymphocytes immunized with PEAMT-like polypeptides such as those set forth in SEQ ID NO: 7, 8, 9, 10, 11 and/or 12.

In addition, antibodies can be engineered, e.g., to produce a single chain antibody (see, for example, Colcher et al. (1999) *Ann N Y Acad Sci* 880: 263-280; and Reiter (1996) *Clin Cancer Res* 2: 245-252). In still another implementation, antibodies are selected or modified based on screening procedures, e.g., by screening antibodies or fragments thereof from a phage display library.

Antibodies of the present invention have a variety of important uses within the scope of this invention. For example, such antibodies can be used: (i) as therapeutic compounds to passively immunize an animal in order to protect the animal from nematodes susceptible to antibody treatment; (ii) as reagents in experimental assays to detect presence of nematodes; (iii) as tools to screen for expression of the gene product in nematodes, animals, fungi, bacteria, and plants; and/or (iv) as a purification tool of PEAMT-like protein; (v) as PEAMT inhibitors/activators that can be expressed or introduced into plants or animals for therapeutic purposes.

An antibody against a phosphoethanolamine n-methyltransferase-like protein can be produced in a plant cell, e.g., in a transgenic plant or in culture (see, e.g., U.S. Patent No. 6,080,560).

Antibodies that specifically recognize a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* PEAMT-like proteins can be used to identify *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* nematodes, and, thus, can be used to monitor a disease caused by *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis.*

Nucleic Acids Agents

Also featured are isolated nucleic acids that are antisense to nucleic acids encoding nematode phosphoethanolamine n-methyltransferase-like proteins. An "antisense" nucleic acid includes a sequence that is complementary to the coding strand of a nucleic acid encoding a PEAMT-like protein. The complementarity can be in a coding region of the coding strand or in a noncoding region, e.g., a 5' or 3' untranslated region, e.g., the translation start site. The antisense nucleic acid can be produced from a cellular promoter (e.g., a RNA polymerase II or III promoter), or can be introduced into a cell, e.g., using a liposome. For example, the antisense nucleic acid can be a synthetic oligonucleotide having a length of about 10, 15, 20, 30, 40, 50, 75, 90, 120 or more nucleotides in length.

An antisense nucleic acid can be synthesized chemically or produced using enzymatic reagents, e.g., a ligase. An antisense nucleic acid can also incorporate modified nucleotides, and artificial backbone structures, e.g., phosphorothioate derivative, and acridine substituted nucleotides.

Ribozymes. The antisense nucleic acid can be a ribozyme. The ribozyme can be designed to specifically cleave RNA, e.g., a PEAMT-like mRNA. Methods for designing such ribozymes are described in U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591. For example, the ribozyme can be a derivative of *Tetrahymena* L-19 IVS RNA in which the nucleotide sequence of the active site is modified to be complementary to a PEAMT-like nucleic acid (see, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742).

Peptide Nucleic acid (PNA). An antisense agent directed against an phosphoethanolamine n-methyltransferase-like nucleic acid can be a peptide nucleic acid (PNA). *See* Hyrup et al. (1996) *Bioorganic & Medicinal Chemistry* 4: 5-23) for methods and a description of the replacement of the deoxyribose phosphate backbone for a pseudopeptide backbone. A PNA can specifically hybridize to DNA and RNA under conditions of low

ionic strength as a result of its electrostatic properties. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996) *supra* and Perry-O'Keefe et al. *Proc. Natl. Acad. Sci.* 93: 14670-14675.

RNA Mediated Interference (RNAi). A double stranded RNA (dsRNA) molecule can be used to inactivate a phosphoethanolamine n-methyltransferase-like gene in a cell by a process known as RNA mediated-interference (RNAi; e.g., Fire et al. (1998) *Nature* 391:806-811, and Gönczy et al. (2000) *Nature* 408:331-336). The dsRNA molecule can have the nucleotide sequence of a PEAMT-like nucleic acid described herein or a fragment thereof. The molecule can be injected into a cell, or a syncytium, e.g., a nematode gonad as described in Fire et al., *supra*.

Screening Assays

Another embodiment of the present invention is a method of identifying a compound capable of altering (e.g., inhibiting or enhancing) the activity of PEAMT-like molecules. This method, also referred to as a "screening assay," herein, includes, but is not limited to, the following procedure: (i) contacting an isolated PEAMT-like protein with a test inhibitory compound under conditions in which, in the absence of the test compound, the protein has PEAMT-like activity; and (ii) determining if the test compound alters the PEAMT-like activity. Suitable inhibitors or activators that alter a nematode PEAMT-like activity include compounds that interact directly with a nematode PEAMT-like protein, perhaps but not necessarily, in the active or binding site. They can also interact with other regions of the nematode PEAMT protein by binding to regions outside of the active site or site responsible for regulation, for example, by allosteric interaction.

In one embodiment the *A. suum, H. contortus, M. incognita, M. javanica* or *S. stercoralis* PEAMT is expressed in a yeast or bacterial cell and then purified and screened in a TLC based radioactivity assay (Bolognese & McGraw (2000) *Plant Physiol.* 124(4):1800-13; Nuccio et al. (2000) *J Biol Chem.* 275(19):14095-101; Charron et al. (2002) *Plant Physiol.* 129(1):363-73). ^{14}C-labelled S-adenosyl-methionine (^{14}C-SAM) co-factor is used and the conversion of phosphoethanolamine (pEA), phosphomonomethylethanolamine (pMME), or phosphodimethylethanolamine (pDME) to ^{14}C-labelled pMME, pDME or phosphocholine (pCho) is monitored after TLC separation. Compounds that decrease the

conversion of pEA, pMME or pDME to pMME, pDME or pCho are candidate PEAMT inhibitors.

Compounds. A test compound can be a large or small molecule, for example, an organic compound with a molecular weight of about 100 to 10,000; 200 to 5,000; 200 to 2000; or 200 to 1,000 daltons. A test compound can be any chemical compound, for example, a small organic molecule, a carbohydrate, a lipid, an amino acid, a polypeptide, a nucleoside, a nucleic acid, or a peptide nucleic acid. Small molecules include, but are not limited to, metabolites, metabolic analogues, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds). Compounds and components for synthesis of compounds can be obtained from a commercial chemical supplier, e.g., Sigma-Aldrich Corp. (St. Louis, MO). The test compound or compounds can be naturally occurring, synthetic, or both. A test compound can be the only substance assayed by the method described herein. Alternatively, a collection of test compounds can be assayed either consecutively or concurrently by the methods described herein.

Compounds can also act by allosteric inhibition or directly by preventing the substrate phosphoethanolamine, phosphomonomethylethanolamine, phosphodimethylethanolamine or the cofactor S-adenosylmethionine from binding to the enzyme, and thus, regulating its target, i.e., a phosphoethanolamine n-methyltransferase.

A high-throughput method can be used to screen large libraries of chemicals. Such libraries of candidate compounds can be generated or purchased, e.g., from Chembridge Corp. (San Diego, CA). Libraries can be designed to cover a diverse range of compounds. For example, a library can include 10,000, 50,000, or 100,000 or more unique compounds. Merely by way of illustration, a library can be constructed from heterocycles including pyridines, indoles, quinolines, furans, pyrimidines, triazines, pyrroles, imidazoles, naphthalenes, benzimidazoles, piperidines, pyrazoles, benzoxazoles, pyrrolidines, thiphenes, thiazoles, benzothiazoles, and morpholines. A library can be designed and synthesized to cover such classes of chemicals, e.g., as described in DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909-6913; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Zuckermann et al. (1994) *J. Med. Chem.* 37:2678-2685; Cho et al. (1993) *Science* 261:1303-

1305; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al. (1994) *J. Med. Chem.* 37:1233-1251.

Organism-based Assays. Organisms can be grown in microtiter plates, e.g., 6-well, 32-well, 64-well, 96-well, 384-well plates.

In one embodiment, the organism is a nematode. The nematodes can be genetically modified. Non-limiting examples of such modified nematodes include: 1) nematodes or nematode cells (*A. suum, H. contortus, M. incognita, M. javanica, S. stercoralis*, and/or *C. elegans*) having one or more PEAMT-like genes inactivated (e.g., using RNA mediated interference); 2) nematodes or nematode cells expressing a heterologous PEAMT-like gene, e.g., an PEAMT-like gene from another species; and 3) nematodes or nematode cells having one or more endogenous PEAMT-like genes inactivated and expressing a heterologous PEAMT-like gene, e.g., a *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* PEAMT-like gene as described herein.

A plurality of candidate compounds, e.g., a combinatorial library, can be screened. The library can be provided in a format that is amenable for robotic manipulation, e.g., in microtitre plates. Compounds can be added to the wells of the microtiter plates. Following compound addition and incubation, viability and/or reproductive properties of the nematodes or nematode cells are monitored.

The compounds can also be pooled, and the pools tested. Positive pools are split for subsequent analysis. Regardless of the method, compounds that decrease the viability or reproductive ability of nematodes, nematode cells, or progeny of the nematodes are considered lead compounds.

In another embodiment, the compounds can be tested on a microorganism or a eukaryotic or mammalian cell line, e.g., rabbit skin cells, Chinese hamster ovary cells (CHO), and/or Hela cells. For example, CHO cells absent for PEAMT-like genes, but expressing a nematode PEAMT-like gene can be used. The generation of such strains is routine in the art. As described above for nematodes and nematode cells, the cell lines can be grown in microtitre plates, each well having a different candidate compound or pool of candidate compounds. Growth is monitored during or after the assay to determine if the compound or pool of compounds is a modulator of a nematode PEAMT-like polypeptide.

***In Vitro* Activity Assays.** The screening assay can be an *in vitro* activity assay. For example, a nematode phosphoethanolamine n-methyltransferase-like polypeptide can be purified as described above. The polypeptide can be disposed in an assay container, e.g., a well of a microtitre plate. A candidate compound can be added to the assay container, and the PEAMT-like activity is measured. Optionally, the activity is compared to the activity measured in a control container in which no candidate compound is disposed or in which an inert or non-functional compound is disposed

***In Vitro* Binding Assays.** The screening assay can also be a cell-free binding assay, e.g., an assay to identify compounds that bind a nematode PEAMT-like polypeptide. For example, a nematode PEAMT-like polypeptide can be purified and labeled. The labeled polypeptide is contacted to beads; each bead has a tag detectable by mass spectroscopy, and test compound, e.g., a compound synthesized by combinatorial chemical methods. Beads to which the labeled polypeptide is bound are identified and analyzed by mass spectroscopy. The beads can be generated using "split-and-pool" synthesis. The method can further include a second assay to determine if the compound alters the activity of the PEAMT-like polypeptide.

Optimization of a Compound. Once a lead compound has been identified, standard principles of medicinal chemistry can be used to produce derivatives of the compound. Derivatives can be screened for improved pharmacological properties, for example, efficacy, pharmacokinetics, stability, solubility, and clearance. The moieties responsible for a compound's activity in the above-described assays can be delineated by examination of structure-activity relationships (SAR) as is commonly practiced in the art. One can modify moieties on a lead compound and measure the effects of the modification on the efficacy of the compound to thereby produce derivatives with increased potency. For an example, see Nagarajan et al. (1988) *J. Antibiot.* 41:1430-1438. A modification can include N-acylation, amination, amidation, oxidation, reduction, alkylation, esterification, and hydroxylation. Furthermore, if the biochemical target of the lead compound is known or determined, the structure of the target and the lead compound can inform the design and optimization of derivatives. Molecular modeling software to do this is commercially available (e.g., Molecular Simulations, Inc.). "SAR by NMR," as described in Shuker et al. (1996) *Science*

274:1531-1534, can be used to design ligands with increased affinity, by joining lower-affinity ligands.

A preferred compound is one that interferes with the function of a phosphoethanolamine n-methyltransferase-like polypeptide and that is not substantially toxic to plants, animals, or humans. By "not substantially toxic" it is meant that the compound does not substantially affect the respective animal, or human PEAMT proteins or phosphoethanolamine n-methyltransferase activity. Thus, particularly desirable inhibitors of *A. suum, H. contortus, M. incognita, M. javanica* and/or *S. stercoralis* PEAMT do not substantially inhibit non-nematode PEAMT-like polypeptides or phosphatidylethanolamine n-methyltransferase activity of vertebrates, e.g., humans for example. Other desirable compounds do not substantially inhibit to phosphoethanolamine n-methyltransferase activity of plants such as tomato (GenBank® Identification No: 12584943), spinach (GenBank® Identification No: 7407189), or wheat (GenBank® Identification No: 17887465).

Standard pharmaceutical procedures can be used to assess the toxicity and therapeutic efficacy of a modulator of a PEAMT-like activity. The LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population can be measured in cell cultures, experimental plants (e.g., in laboratory or field studies), or experimental animals. Optionally, a therapeutic index can be determined which is expressed as the ratio: LD50/ED50. High therapeutic indices are indicative of a compound being an effective PEAMT-like inhibitor, while not causing undue toxicity or side effects to a subject (e.g., a host plant or host animal).

Alternatively, the ability of a candidate compound to modulate a non-nematode phosphoethanolamine n-methyltransferase-like polypeptide is assayed, e.g., by a method described herein. For example, the inhibition constant of a candidate compound for a mammalian PEAMT-like polypeptide can be measured and compared to the inhibition constant for a nematode PEAMT-like polypeptide.

The aforementioned analyses can be used to identify and/or design a modulator with specificity for nematode phosphoethanolamine n-methyltransferase-like polypeptide over vertebrate or other animal (e.g., mammalian) phosphatidylethanolamine n-methyltransferase -like polypeptides. Suitable nematodes to target are any nematodes with the PEAMT-like

proteins or proteins that can be targeted by a compound that otherwise inhibits, reduces, activates, or generally affects the activity of nematode PEAMT proteins.

Inhibitors of nematode phosphoethanolamine n-methyltransferase-like proteins can also be used to identify phosphoethanolamine n-methyltransferase-like proteins in the nematode or other organisms using procedures known in the art, such as affinity chromatography. For example, a specific antibody may be linked to a resin and a nematode extract passed over the resin, allowing any PEAMT-like proteins that bind the antibody to bind the resin. Subsequent biochemical techniques familiar to those skilled in the art can be performed to purify and identify bound PEAMT-like proteins.

Agricultural Compositions

A compound that is identified as a phosphoethanolamine n-methyltransferase-like polypeptide inhibitor can be formulated as a composition that is applied to plants, soil, or seeds in order to confer nematode resistance. The composition can be prepared in a solution, e.g., an aqueous solution, at a concentration from about 0.005% to 10%, or about 0.01% to 1%, or about 0.1% to 0.5% by weight. The solution can include an organic solvent, e.g., glycerol or ethanol. The composition can be formulated with one or more agriculturally acceptable carriers. Agricultural carriers can include: clay, talc, bentonite, diatomaceous earth, kaolin, silica, benzene, xylene, toluene, kerosene, N-methylpyrrolidone, alcohols (methanol, ethanol, isopropanol, n-butanol, ethylene glycol, propylene glycol, and the like), and ketones (acetone, methylethyl ketone, cyclohexanone, and the like). The formulation can optionally further include stabilizers, spreading agents, wetting extenders, dispersing agents, sticking agents, disintegrators, and other additives, and can be prepared as a liquid, a water-soluble solid (e.g., tablet, powder or granule), or a paste.

Prior to application, the solution can be combined with another desired composition such as another anthelmintic agent, germicide, fertilizer, plant growth regulator and the like. The solution may be applied to the plant tissue, for example, by spraying, e.g., with an atomizer, by drenching, by pasting, or by manual application, e.g., with a sponge. The solution can also be distributed from an airborne source, e.g., an aircraft or other aerial object, e.g., a fixture mounted with an apparatus for spraying the solution, the fixture being of sufficient height to distribute the solution to the desired plant tissues. Alternatively, the composition can be applied to plant tissue from a volatile or airborne source. The source is

placed in the vicinity of the plant tissue and the composition is dispersed by diffusion through the atmosphere. The source and the plant tissue to be contacted can be enclosed in an incubator, growth chamber, or greenhouse, or can be in sufficient proximity that they can be outdoors.

If the composition is distributed systemically thorough the plant, the composition can be applied to tissues other than the leaves, e.g., to the stems or roots. Thus, the composition can be distributed by irrigation. The composition can also be injected directly into roots or stems.

A skilled artisan would be able to determine an appropriate dosage for formulation of the active ingredient of the composition. For example, the ED50 can be determined as described above from experimental data. The data can be obtained by experimentally varying the dose of the active ingredient to identify a dosage effective for killing a nematode, while not causing toxicity in the host plant or host animal (i.e. non-nematode animal).

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS: